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THE ORGANIC MATTER OF THE SOIL: I. SOME DATA ON HUMUS, HUMUS CARBON AND HUMUS NITROGEN

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INTRODUCTION

Since the time of Liebig, the nature of the organic matter of the soil has interested chemists, and every year sees extensive contributions to the voluminous literature. It would be far beyond the scope of the present paper to attempt a complete review of the literature and the bibliography will therefore be limited as much as possible to those papers which deal directly with the problems at hand.

Briefly the problems may be stated as follows:

1. Is the humus, the ammonia or alkaline hydroxide extract of soils, a typical soil product, formed in the soil by the action of bacteria and fungi, or can similar extracts be obtained from unchanged vegetable material?

2. Does the "humus," the "matière noire" of Grandeau, consist entirely of a black compound or compounds, or does it contain in addition a greater or less proportion of colorless substances whose presence is masked by a black pigment?

3. Does a 4 per cent sodium hydroxide solution extract the same substances from a soil as a 4 per cent ammonium hydroxide solution, and, if the same substances are extracted, is the same quantity extracted by each solvent?

4. Is the soil nitrogen present in a form different from that in which the nitrogen occurs in plant materials, or does the nitrogen of the soil exhibit the same solubilities as vegetable nitrogen?

¹ The work embodied in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soil Chemist.

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In regard to the first and second questions: Hilgard's views on the nature of the ammonia extract of soils and his statements as to the normal processes of humification (17, p. 123-4) have been very widely accepted. He regards the humus of soils as a definite soil product, formed from vegetable material in the soil under the influence of fungous and bacterial growths: this conversion being most efficiently carried out in the presence of only a moderate amount of moisture, under the influence of a more or less rapid circulation of air, and in the presence of calcium carbonate to neutralize any acids which may be formed. Under these conditions the vegetable substance is converted into black, neutral, insoluble humus compounds.

Hilgard believed that the nitrogen contained in the "humified" portion of the soil organic matter was the only important part of the soil nitrogen which could be converted into nitrates under the influence of the nitrifying bacteria and that the nitrogen of plant debris must first pass through these intermediate stages before being available for the use of the higher plants. His views of the persistence of plant materials in soils are contained in the following statement:

"As a matter of course, the several organic compounds contained in plants may continue to exist in soils for some time, varying according to conditions of temperature and moisture. Thus dextrin, glucose, and even lecithin and nuclein have been reported to be found. The activity of the numerous fungous and bacterial ferments under favoring conditions, will, of course, limit the continued existence of such compounds somewhat narrowly, so that they can hardly be considered as active soil ingredients save in so far as they favor the development of the bacterial flora."

Grandeau (13) elaborated a method for the estimation of the "matière noire" of soils by first leaching the soil with dilute acid in order to set the humus free from its combination with the alkaline earths, removing the excess of acid by washing with water, then *moistening* the soil with ammonia and allowing it to stand for a short time (three to four hours, cf. Grandeau 1877, [14] p. 149), after which the humus solution was displaced by repeated washings with ammonical water. The *dark brown* solution so obtained was evaporated to dryness in platinum, weighed, ashed and the amount of "matière noire" and of ash recorded. Grandeau regarded the humus ash as an integral part of the humus.

Grandeau's method, more or less modified, is the basis for all modern methods for the estimation of humus, and Hilgard (17, p. 132) expressed a view, generally accepted in this country, of the nature of the substances dissolved, in the following statements:

"It has been usual to determine the amount of humus in soils by means of (dry or wet) combustion, calculating the humus from the

carbonic dioxid so formed, while measuring the nitrogen gas directly. But in this process the entire organic matter of the soil, humified and unhumified, is indiscriminately included; and it is wholly uncertain to what extent the latter will ultimately become humus, from the nitrification of which plants are presumed chiefly to derive their nitrogen. In order to obtain definite results, the actual, functional humus must be extracted from the soil mass by some solvent which discriminates between the humified and unhumified organic matter. This cannot be done by direct extraction with caustic soda or potash, which inevitably dissolve unhumified matters and tend to expel ammonia from the humus; besides themselves acting as humifiers. *The only method now known which accomplishes this separation, practically excluding the unhumified while fully dissolving the humified matter is that of Grandeau.*"¹

In view of the well known ability of ammonia solutions to dissolve organic compounds, it seemed highly improbable that ammonium hydroxide would dissolve only the "humified" portion. Experiments were therefore instituted in which *unleached* soils were extracted with 4 per cent ammonium hydroxide *and in every instance* a solution was obtained which was relatively colorless, but which, nevertheless, contained a very considerable amount of dissolved organic compounds. When this solution was evaporated to dryness on a water bath the compounds were more or less oxidized and a brown residue remained, in some respects resembling typical humus but lacking the black lustrous color. If the evaporation is carried out at 60° C. *in vacuo*, this browning can be largely prevented.

If one speculates on the nature of the soil organic matter, it becomes obvious that the variety of compounds which are present in a soil is limited only by those compounds which were present in the plants growing upon the soil, *plus* those compounds which compose the bodies of bacteria and protozoa, *plus* the compounds contained in the soil fungi, *plus* all of the various compounds which may be formed from the above sources by decay, oxidation, and all of the intricate chemical reactions which take place in converting dead organic material, either into living protoplasm, on the one hand, or into water, carbon dioxide and nitrogen on the other. Undoubtedly these organic compounds are not the product of "humification" but are derived from unchanged plant material, from protozoa, or from bacteria.

In certain experiments, unhumified vegetable materials yielded in some instances as much as 50 per cent of "humus," if by "humus" we mean the organic matter soluble in 4 per cent ammonia. In some of the experiments with unhumified vegetable materials the ammonia ex-

¹ Italics by the author.

tract exhibited all of the properties of true "humus" solutions excepting that it lacked the true black color, being rather a very dark brown. It, therefore, appears extremely improbable that more than a small percentage of the humus, the "matière noire" extracted by Grandeau's method, is actually *black*. Recently the author has succeeded in isolating the black *soil pigment* and found that it is present in relatively small amounts.

In order to answer the third and fourth questions it was necessary to make a large series of experiments. These questions are important since the nitrogen content of "humus" has been determined by the method of Hilgard and Jaffa (18) in which sodium hydroxide replaces the ammonium hydroxide of the humus extraction. Alway and Bishop (1) recently called attention to the fact that "the correctness of the assumption that the ammonia solution dissolves the same compounds or the same proportion of total nitrogen as the alkaline hydroxides is open to serious question A determination of the organic carbon in both solvents should be made, and if this is not the same, the nitrogen in the alkaline hydroxide solution is not to be regarded as that corresponding to the whole of the organic matter dissolved by the ammonia."

Such a study is reported in this paper, and it is found that not only is more carbon extracted by the alkaline hydroxide than by the ammonia, but that practically all of the nitrogen that is extractable from the soil after leaching with 1 per cent HCl is also extractable *without previous leaching*. Likewise none of the *soil pigment* is soluble in 4 per cent NaOH without previously leaching the soil, *and but very little is soluble in 4 per cent NaOH after leaching*, whereas, it is readily soluble in 4 per cent ammonia, after leaching with acid. In other words, 4 per cent sodium hydroxide does not extract the same compounds, nor the same proportion of compounds that are extracted by 4 per cent ammonium hydroxide, and the nitrogen figures obtained on sodium hydroxide extracts cannot be properly correlated with "humus" obtained by ammonia extraction.

EXPERIMENTAL

I. The Analytical Methods

The Preparation of the Soil Extracts and the Estimation of "Humus". Throughout this work only air-dry soils and vegetable materials were used. When one remembers the ease with which plant saps are decomposed by heat, it becomes exceedingly questionable whether the extracts of soils dried at 100° C. or 110° C. would contain the same compounds as those extracts made from unheated soils. Schreiner and Lathrop (26), Seaver and Clark (27, 28), Lyon and Bizzell (21), as well as numerous other investigators have made studies

on heated soils and, almost without exception, the finding is that the solubility of the organic matter is greatly altered by heating.

Fifteen gm. of the air-dry soil or 5 gm. of the air-dry vegetable material, either with or without previous leaching with 1 per cent HCl as the case might be, were placed in a 1-liter bottle, 750 c.c. of 4 per cent NaOH, 2 per cent NaOH, or 4 per cent NH_4OH added, the bottle tightly stoppered with a rubber stopper and shaken continuously for one week in a shaking machine, in which the bottles were rotated "wheel fashion" so that *the soil was continually falling through the solution*. The speed was regulated so that the bottles made about ten revolutions per minute. At the end of the week the bottles were removed from the shaking machine and the soil was allowed to settle. In three or four days the soil had completely settled in those bottles to which sodium hydroxide had been added, and the clear supernatant liquid could be siphoned off for analysis.

In those bottles, however, to which ammonium hydroxide had been added, the soil did not settle readily. The following procedure was therefore adopted: Approximately 200 c.c. of the solution was siphoned off into a glass-stoppered cylinder and 3 gm. of K_2SO_4 added to precipitate the clay. After the clay had all settled out, leaving the supernatant liquid clear, portions of 50 c.c. were removed and used for the determination of organic carbon (see later). The remaining solution was siphoned off into a second glass-stoppered cylinder and 2 gm. of ammonium carbonate added to precipitate the clay as in Rather's (25) method. After all of the clay had precipitated, the solution was filtered and portions of from 150 to 250 c.c. were evaporated to dryness on a steam bath in weighed quartz dishes, then dried at 105°C ., and ashed in an electric muffle. The loss on ignition was recorded as "humus" and the residue as "humus ash."

Undoubtedly in these determinations a higher humus content is shown than would be found by using any of the standard methods, such as Rather's (25), Hilgard's (16), the Moores-Hampton (23), etc., because of the greater length of time in contact with the ammonium hydroxide and because of the more thorough and constant shaking. However, *the results are all comparable with each other* and the carbon extracted by the ammonium hydroxide was extracted in exactly the same way as in the case of sodium hydroxide. It is impossible to "shake occasionally for 24 hours" as in the "official" (6) and Rather (25) methods and always have exactly the same conditions. Then again it was desired to approach as nearly as practicable to the maximum solubility of the organic matter. Had the bottles been shaken for two weeks instead of for one week, there would have been an increase in the amount of humus and carbon dissolved, but a longer time seemed impracticable.

One noteworthy difference was observed between those soils which were treated in the unleached condition, and those previously leached with 1 per cent HCl: *i. e.*, the soil settled rapidly from the solution in the case of the leached soils plus NaOH but only slowly in the case of the unleached soil plus NaOH. The clay was flocculated within a few minutes on adding ammonium carbonate to the ammonia extract of the leached soils, but it often happened that no flocculation of the clay would take place for a week or more when ammonium carbonate was added to the extracts from the unleached soil. However, once the clay began to flocculate in these extracts, it came down quite rapidly. This suggests a very different colloidal state of the clay in the two instances.

The Determination of Organic Carbon. Organic carbon was determined by wet combustion in the apparatus shown in figure 1. No novel features are claimed for this apparatus, since it is a combination of the best parts of several wet-combustion methods. However, in view of the accuracy of the results which can be obtained, it is thought worth while to describe the apparatus in some detail.

"A" contains a 1:1 solution of KOH in order to remove all CO_2 from the air aspirated through the apparatus. "E" contains a saturated solution of silver sulphate in 5 per cent H_2SO_4 , in order to remove any traces of hydrochloric acid or chlorine which might be present. It is best to add a considerable excess of solid silver sulphate, allowing this to lie in the bottom of the U tube so as to have at all times a saturated solution. "F" is a Schmitz drying tube containing concentrated sulphuric acid. "G" contains anhydrous, granular, calcium chloride. "H" is the potash bulb filled with 1:1 KOH solution, and "I" contains soda lime, to protect the potash bulb from moisture and carbon dioxide from the outside air.

Truog (30), recently criticized the weighing of CO_2 in a potash bulb because of the error which might be introduced, due to the small increase in weight in a relatively heavy piece of apparatus, and also since the drying materials are not the same at each end of the potash bulb. The first criticism can be obviated by always weighing the potash bulb against a similar bulb used as a counterpoise. In this manner barometric and temperature changes are compensated. All weights were made with the use of a second potash bulb for a counterpoise, the weight of the counterpoise being within 2 or 3 gm. of the potash bulb used.

The second criticism has been met by fastening a small tube filled with anhydrous calcium chloride upon the intake tube of the "Gomberg" and weighing this as an integral part of the potash bulb. In this manner the solution of KOH is protected at both outlets from evaporation. By these modifications the weighing of CO_2 becomes as accurate as, or more accurate than, any titrametric method.

"D" is an electric furnace containing a copper oxide spiral which is maintained at a dull red heat during the determination, as well as a silver spiral near the exit end of the furnace tube. This is thought necessary in order to burn any carbon monoxide or volatile organic compounds which may be formed by the chromic acid oxidation. The furnace can be easily constructed in the laboratory. It consists of a silica combustion tube 15 inches long with an inner diameter of $\frac{3}{8}$ of an inch, glazed inside and out. Around the middle of this tube is wound approximately 50 feet of No. 27 "nichrom" wire, which had been previously coiled into a spiral of about 5 mm. diameter. The nichrom winding should cover about 6 inches of the silica tube. The windings

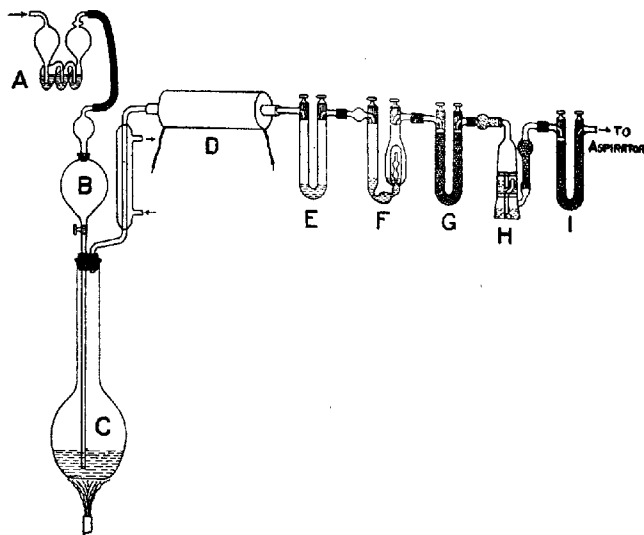


Fig. 1.—Apparatus for the determination of organic carbon by wet combustion.

are embedded in "alundum" cement, or in a cement made from equal parts of powdered moulding clay and finely powdered quartz. After drying at 110° C. until all moisture is removed, the cement is hardened by heating the coil by means of an electric current. A very convenient outer jacket for the furnace can be made from a porous clay cup, 3 inches in diameter by 7 inches high, such as is used in wet electric batteries. The space between the cement covering the wire, and the wall of the porous cup is tightly packed with loose asbestos fiber and the open end of the cup closed with a piece of asbestos board.

A furnace of these dimensions can be operated on a 220-volt circuit in series with a rheostat made from 110-volt electric light bulbs. The

rheostat for my own furnace consists of four 25-watt tungsten, and one 55-watt carbon bulbs, connected in parallel. The furnace heats to dull red in about 15 minutes when all lights are burning, and is kept at that temperature indefinitely by burning the carbon and two of the tungsten lamps, or a current consumption of 105 watts. This furnace has been run practically 8 hours a day for a year and a half and shows no sign of deterioration.

A carbon determination is made as follows: 50 c.c. of soil extract (or 1 to 2 gm. of soil plus 50 c.c. CO_2 -free water) is placed in the 200-c.c. long-necked Kjeldahl flask "C," 5 gm. of potassium dichromate is added and the flask connected with the condenser and separatory funnel; 75 c.c. of concentrated H_2SO_4 is then slowly run in from the separatory funnel "B", in the top of which is a ground glass connection to which can be fastened the rubber tube leading to the potash guard bulb "A". A rubber stopper cannot be used in the top of the separatory funnel because of the danger of dissolving some rubber in the sulphuric acid.

After all of the sulphuric acid has been run in, the contents of the flask are mixed by gentle shaking and the aspiration is started. It has been found advisable to aspirate about $2\frac{1}{2}$ liters of air through the apparatus, the entire time of aspiration being about $2\frac{1}{2}$ hours. During the first two-thirds of the aspiration the contents of the flask are kept just below the boiling temperature by means of a low flame. The flask cools during the last one-third of the aspiration so that a second determination can be started at once.

By this method we obtain total carbon, *i. e.*, both organic carbon and inorganic carbonates, and the organic carbon is obtained by making a separate determination of the carbonate CO_2 and subtracting this from the total CO_2 .

After some work had been done it was found that it was impossible accurately to determine carbon in a 4 per cent solution of ammonium hydroxide without previously evaporating off the ammonia. Ames and Gaither (5) report the analysis of 4 per cent ammonia extracts of soils by chromic oxidation and obtain an average of 1.807 per cent carbon for the extracts evaporated to dryness and 1.970 per cent carbon for the extracts not evaporated. They suggest that this difference "can be accounted for by the loss of volatile and easily oxidizable carbonaceous matter through evaporation on the steam bath." If this were the case, one would think that a similar, and greater loss would be observed when soils are dried at 110°C ., but such is not the case. When 50 c.c. of 4 per cent ammonia is oxidized by chromic acid the potash bulb may increase as much as 0.0260 gm., whereas when this same solution is evaporated to dryness in the Kjeldahl flask *in vacuo* at 60°C ., there is a "blank" not greater than 0.0010 gm. CO_2 . The factors involved in this

behavior have not been ascertained but it is possible that ammonium cyanide or some of the aliphatic amines are present. Nitric acid, and oxides of nitrogen are formed to some extent but they are not important factors since the KOH of the potash bulb gave only a faint trace of nitrates with diphenylamine after 8 or 10 oxidations of 50 c.c. of 4 per cent ammonia.

In all of the following work the ammonia solutions were evaporated to dryness *in vacuo* at 60° C. in the Kjeldahl flask in which they were subsequently oxidized, 50 c.c. distilled water, free from CO₂, added and treated with chromic acid as above.

One should here present some data showing the accuracy of the wet-combustion method. A few determinations of organic carbon in soils are shown in Table I. It is necessary, however, to reserve the really conclusive proof for the second paper in this series. It suffices to say, however, that *organic carbon was determined on the original soil, then on sixteen consecutive soil extracts and, lastly, on the residual soil, and a recovery of 101.8 per cent of the original carbon was effected.* The 1.8 per cent increase represented an error of only 0.00032 gm. carbon per determination.

TABLE I
THE AGREEMENT BETWEEN ORGANIC CARBON DETERMINATIONS IN SOILS
OXIDIZED BY WET COMBUSTION AND BY IGNITION WITH
COPPER OXIDE IN OXYGEN

Soil	Per cent Organic Carbon by Wet Combustion			Per cent Organic Carbon by Copper Oxide in Oxygen
	I	II	Average	
Carrington Silt Loam ..	4.757	4.709	4.733	4.701
Hempstead Silt Loam...	3.379	3.367	3.373	3.324
Fargo Silt Loam.....	9.959	10.080	10.020	10.030
Sphagnum-covered Peat..	49.290	49.340	49.320	49.010
Forest-covered Loess....	1.637	1.639	1.638	1.612

The Determination of Inorganic Carbon. The determination of CO₂ derived from carbonates is carried out in an apparatus exactly like that used for total carbon, with the exception that the electric furnace is omitted.

The soil extract is placed in the Kjeldahl flask and 10 c.c. of 85 per cent phosphoric acid added through the separatory funnel. Aspiration is started and the contents of the flask heated to about 80° C. to ensure complete action, and then allowed to cool. In the case of soils from 5 to 10 gm. of soil are taken, 50 c.c. of CO₂-free water added, and the carbonates decomposed with 25 c.c. of 5 per cent (by weight) HCl. Otherwise the conditions are the same as for the soil solutions.

The Determination of Nitrogen. Nitrogen was determined on the soils and soil extracts in the usual manner, 25 to 35 c.c. H_2SO_4 , 10 gm. K_2SO_4 , and a crystal of CuSO_4 being used. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without necessitating a calculation.

II. The Samples Analysed

Seventeen samples of soil or vegetable materials were analysed. The soils used were selected from those in stock in the laboratory and recently collected in connection with other investigations. All were from Minnesota with the exception of No. 6 which was from California and No. 8 from Nebraska. The soils are named in accordance with the surveys of the Bureau of Soils of the U. S. Department of Agriculture when such surveys are available.

1. *Fargo Clay Loam.* The sample analysed consisted of a composite made from 144 borings taken to a depth of 8 inches from a 20-acre field on the Northwest Sub-station farm, Crookston, Minn. The sample was highly calcareous. This soil type has been described by Mangum and Schroeder (22). Fifteen gm. of air-dry soil containing 3.92 per cent of moisture were used in all the extractions.

2. *Fargo Silt Loam.* This sample was a composite made from 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields near Nerstrand, Rice Co., Minn. The sample had a neutral reaction. The soil type has been described by Burke and Kolbe (10). The air-dry soil contained 14.89 per cent of moisture. Fifteen gm. portions were used for the preparation of the extracts.

3. *Carrington Silt Loam.* This soil sample was likewise a composite of 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields near Nerstrand, Rice Co., Minnesota. The sample was strongly acid. The soil type is described by Burke and Kolbe (10). Fifteen gm. of air-dry soil were used in preparing the extracts, the moisture content being 6.22 per cent.

4. The *Prairie-covered Loess* sample consisted of a composite from 50 borings taken to a depth of 1 foot, 10 borings being taken from each of 5 virgin fields near Luverne, Rock Co., Minn. The sample was somewhat calcareous. Fifteen-gm. portions of air-dry soil were taken for analysis, the moisture content being 7.89 per cent.

5. The *Forest-covered Loess* sample was taken from 5 virgin fields near Caledonia, Huston Co., Minn. Ten borings to a depth of 1 foot were taken from each field and equal weights from each boring combined in the composite sample. The sample was strongly acid. Fifteen gm. of air-dry soil were used in each experiment, the moisture content being 1.87 per cent.

6. The "*Berkeley Adobe*". It seemed desirable to include in these experiments a sample of a truly arid soil. For this I used a composite of two samples from Berkeley, California, collected by Professor Alway, and by him kindly placed at my disposal. They were the soils "A" and "B" on which some analyses have been reported by Alway and Bishop (1). They give the history of the samples as follows:

"Samples A and B were both taken from the high hill just east of the buildings on the grounds of the University of California. Sample A is a composite of 20 samples from near the summit, and B of the same number from the lighter colored soil to the west, below the summit." The samples were taken to a depth of 3 inches from virgin soil. In order to obtain a sufficiently large sample it was necessary to combine all that remained of the two samples which differed considerably in weight. Unfortunately the samples were not weighed before combining.

The soil analysed was slightly acid and contained 2.75 per cent of moisture. Fifteen-gm. portions were used in preparing the extracts.

7. *Hempstead Silt Loam*. A composite sample from 36 borings to a depth of 6 inches was taken from 12 plots on the Minnesota Agricultural Experiment Station farm, St. Paul. No commercial fertilizer had been applied, but the land had long been under cultivation. The soil type has been described by Smith and Kirk (29). The sample was strongly acid. Fifteen-gm. portions of air-dry soil containing 3.07 per cent of moisture were used in the different experiments.

8. *Marshall Silt Loam*. This soil was part of a bulk sample taken to a depth of 6 inches from a long cultivated (40 years) field on the farm of the Nebraska Agricultural Experiment Station at Lincoln. The soil type has been described by Burgess and Worthen, (9). The sample was slightly acid, and contained 2.83 per cent of moisture. Fifteen-gm. portions were used in the experiments.

9. A sample of strongly acid "*muck*" soil was obtained from a bog about 2 miles from the farm of the Minnesota Agricultural Experiment Station, St. Paul. The sample is a composite of 10 samples taken to a depth of 8 inches, lengthwise of the bog. The vegetation of the bog was largely "cat-tails" and rushes. Portions of air-dry soil weighing 7.5 gm. each, containing 5.60 per cent of moisture were used in the extractions.

10. *Sphagnum-covered peat*. This sample, which was very strongly acid, was prepared from a large bulk sample taken from a sphagnum-covered bog on the experimental farm near Grand Rapids, Minn. The superficial layer of sphagnum and shrubs was first removed and a sample of the underlying peat taken to a depth of 8 inches. The peat was poorly decomposed. The sample was prepared by grinding the material

to a powder in a ball mill. Five-gm. portions, containing 5.90 per cent of moisture were used in the extracts. An analysis of the peat is shown in Table II.

11. A sample of *Calcareous Black Peat* was selected from a large bulk sample taken to a depth of 8 inches from a grass bog near the Minnesota Agricultural Experiment Station farm, St. Paul. The peat was black and well decomposed. The peat was ground to a powder in a ball mill before using. An analysis of the peat is shown in Table II. For the extractions, 5 gm. of material containing 6.40 per cent of moisture were used.

TABLE II
COMPARATIVE "PEAT ANALYSES" ON THE PEATS AND VEGETABLE MATERIALS USED¹

	Per cent Volatile	Per cent Ash	Per cent Insoluble	Per cent CaO	Per cent P ₂ O ₅	Per cent N	Per cent C
"Muck"	26.75	73.25	67.97	0.28	0.11	1.34	14.58
Sphagnum-covered Peat	90.59	9.41	6.12	0.63	0.24	2.00	49.32
Black Peat	78.68	21.32	10.26	4.63	0.47	2.94	42.81
Brown Peat	86.10	13.90	11.37	1.22	0.33	2.82	46.34
Grass from Brown Peat Bog....	92.97	7.03	3.08	0.51	0.24	1.16	47.02
Oat Plants	86.29	13.71	6.15	0.49	1.33	2.13	42.48
Alfalfa Hay	88.47	11.53	1.84	2.47	0.65	3.80	44.26
Sweet Fern Leaves	92.44	7.56	4.88	0.92	0.17	1.59	48.34
Oak Leaves	94.13	5.87	0.66	2.26	0.27	1.00	48.63

¹ The analyses were made according to the method of the Bremen Peat Experiment Station (20), with the exception that all calcium determinations but that of the "Muck" soil were made by the method recently proposed by the author (12). Carbon was determined by wet combustion.

12. A sample of *acid Brown Peat* was selected from a large bulk sample taken from the first 8 inches of a grass bog about a half mile from the buildings of the Minnesota Agricultural Experiment Station. The peat was poorly decomposed, the sample utilized being ground to a powder in a ball mill. An analysis of the peat is shown in Table II. Five-gm. portions containing 11.31 per cent of moisture, were taken for the extraction experiments.

13. A sample of the *grass growing on the Brown Peat Bog* was secured in September, ground to a powder in a ball mill, and used for analysis. This sample, tested by methods for "soil acidity," showed a strongly acid reaction. Five-gm. portions containing 8.50 per cent of moisture were used in the extractions. A "peat analysis" on the grass is shown in Table II.

14-17. A sample of *ripe oat plants* (straw and grain, without the roots) was ground to a powder in the ball mill and used for comparative analyses. Likewise samples of *alfalfa hay*, *sweet fern leaves* (dry and ready to fall) and *oak leaves* (*Quercus coccinea*, Muench) which

had remained on the tree until March, were secured and prepared as above. "Peat analyses" are shown in Table II. All samples showed strong acidity. Five-gm. portions of each were used for the extractions. The moisture percentages were, oats, 6.40; alfalfa, 6.00; sweet fern, 1.35; and oak leaves 1.95.

III. The Presentation of the Analytical Data

The general plan of the experiments. A series of symbols have been used to designate the treatment to which the soil has been subjected in order to obtain the solution in question. The following symbols will be used throughout this paper: "A" signifying *acid* and "B" signifying *base*.

A(HCl) is the 1 per cent hydrochloric extract of the soil, obtained by leaching the soil on a filter with 1 per cent HCl until no more calcium is removed and then washing with water to the absence of chlorides. This solution was utilized only for the determination of nitrogen.

AB(NH₄OH) is the ordinary black humus solution obtained by first leaching the soil with 1 per cent HCl and subsequent extraction of the humus with 4 per cent NH₄OH solution by the method above described.

AB(NaOH) is obtained exactly as AB(NH₄OH) with the exception that the ammonia solution is replaced by 4 per cent NaOH solution. This solution is not black, but usually an intense brown.

B(NH₄OH) is the 4 per cent ammonia extract obtained without previous leaching of the soil with 1 per cent HCl. This solution is usually only slightly colored, the color varying from very light straw to light brown.

B(NaOH) is similar to B(NH₄OH) with the ammonia replaced by 4 per cent NaOH solution. This solution is usually darker in color than B(NH₄OH) but never becomes *black*, being usually more or less brown.

BA(HCl) is the solution obtained by leaching the residue from B(NaOH) with 1 per cent HCl to the absence of calcium, and further washing with water to the absence of chlorides.

BAB(NaOH) is the extract obtained from the leached soil residue from BA(HCl) when this is shaken for one week with 4 per cent NaOH. This solution is usually darker than B(NaOH) or BA(NaOH) but is always brown, never black.

BAB + H₂O The soil residue from BAB(NaOH) is drained on a hardened filter until practically all of solution BAB(NaOH) has been removed. The soil residue, still alkaline from some adhering sodium hydroxide, is added to 750 c.c. of distilled water and the mixture shaken for one week. The solution obtained is designated BAB + H₂O. This solution, with soils, is always *jet black*, when in volume and contains the

true soil pigment which can be precipitated from this solution by the addition of NaOH equal to 4 per cent concentration, or by acidification.

In practically all of the experiments, the soil extracts were prepared in duplicate so that in the following tables the total carbon percentages represent the average of two closely agreeing duplicate determinations, the total nitrogen percentages represent the average of three or more closely agreeing duplicate determinations, the humus and humus ash percentages represent two closely agreeing determinations from separate soil extracts, the carbon percentages of the solutions are likewise averages of closely agreeing duplicate determinations from two distinct soil extracts, while the nitrogen percentages are in most cases the average of four determinations, duplicate analyses being made on from 200 to 250 c.c. of solution from each soil extract.

The individual determinations are not recorded in detail because of the excessive amount of space which would be necessary. All percentages are calculated to the basis of oven-dry soil.

Tables III to XVIII give the analytical data obtained. Table XIX shows a summary of these data. Table X varies somewhat from the usual procedure in that the unleached soil was extracted with 4 per cent NaOH *twice* before leaching with acid for BAB (NaOH). It was necessary to present these analyses in order to give some idea of the amount of nitrogen retained in the soil residue. But in the graphs and in the average tables data are taken from another series of analyses made in the usual manner on the same soil (Marshall Silt Loam), so that all averages are correct.

Table XX shows the approximate colors of the solutions obtained. The colors were approximated by placing a portion of the solution in flat porcelain evaporating dishes so that the depth of the liquid was 1 cm. The color of the center of the solution was then matched against the standard color chart facing page 2334 of Funk and Wagnall's (11) "New Standard Dictionary".

Quantitative measurements of the intensity of coloration are given in Tables XXI and XXII. Table XXI shows the *relative coloration within each series* as referred to the ordinary humus solution AB (NH_4OH). Table XXII gives the quantitative relationships determined colorimetrically and photometrically (4), of the standards used in Table XXI, the standard in this instance being a 0.0063 per cent solution of the "soil pigment".¹

In this table is also shown, for comparative purposes, the weight of "humus" actually obtained per unit volume of solution, as well as the calculated "indicated approximate" and "actual" percentages of soil pigment in the humus solution, AB(NH_4OH).

¹ The description of the isolation and properties of this "soil pigment" will comprise the second paper of this series.

TABLE III
A SUMMARY OF THE DATA ON FARGO CLAY LOAM

Total N in Soil	0.250%	C/N
Total Organic C in Soil	2.678%	
Carbonate CO ₃	2.360%	10.72

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.007	2.80
AB (NaOH)	0.153	61.20	1.537	57.40	10.05
AB (NH ₄ OH)	1.423	53.14	2.68	0.56	53.50
B (NH ₄ OH)	0.476	17.77	0.95	0.46	50.10
B (NaOH)	0.127	50.81	0.979	36.56	7.71
BA (HCl)
BAB (NaOH)	0.070	28.00	0.531	19.83	7.59
BAB + H ₂ O	0.028	11.20	0.428	15.98	15.29
Soil Residue	0.034	13.60	*0.740	*27.63	21.76
Total	0.259	103.61	*100.00

* By difference.

TABLE IV
A SUMMARY OF THE DATA ON FARGO SILT LOAM

Total N in Soil	6.823%	C/N
Total Organic C in Soil	10.020%	
Carbonate CO ₃	0.200%	12.17

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.021	2.55	9.77
AB (NaOH)	0.613	74.49	5.990	59.78	52.20
AB (NH ₄ OH)	5.174	51.64	9.91	0.72	48.62
B (NH ₄ OH)	1.774	17.71	3.65	0.46
B (NaOH)	0.584	70.96	4.555	45.46	7.80
BA (HCl)
BAB (NaOH)	0.090	10.93	1.647	16.44	18.30
BAB + H ₂ O	0.080	9.72	1.530	15.27	19.13
Soil Residue	0.087	10.57	*2.288	*22.83	26.29
Total	0.841	102.18	*100.00

* By difference.

TABLE V

A SUMMARY OF THE DATA ON CARRINGTON SILT LOAM

Total N in Soil	0.371%	C/N
Total Organic C in Soil	4.733%	
Carbonate CO ₂	0.090%	12.76

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.009	2.45
AB (NaOH)	0.266	71.69	2.890	61.05	10.86
AB (NH ₄ OH)	2.525	53.34	4.95	0.40	51.01
B (NH ₄ OH)	1.436	30.35	2.86	0.34	50.20
B (NaOH)	0.271	73.05	2.349	49.63	8.67
BA (HCl)
BAB (NaOH)	0.030	8.17	0.653	13.79	21.77
BAB + H ₂ O	0.032	8.71	0.737	15.57	23.03
Soil Residue	0.044	11.99	*0.994	*21.00	22.59
Total	0.383	101.92	*100.00

* By difference.

TABLE VI

A SUMMARY OF THE DATA ON PRAIRIE-COVERED LOESS

Total N in Soil	0.301%	C/N
Total Organic C in Soil	3.704%	
Carbonate CO ₂	0.240%	12.30

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.010	3.32
AB (NaOH)	0.194	64.46	2.049	55.31	10.56
AB (NH ₄ OH)	1.737	46.88	3.40	0.42	51.08
B (NH ₄ OH)	0.702	18.95	1.42	0.33	49.43
B (NaOH)	0.186	61.79	1.548	41.78	8.32
BA (HCl)
BAB (NaOH)	0.049	16.28	0.798	21.53	16.29
BAB + H ₂ O	0.025	8.20	0.500	13.50	20.00
Soil Residue	0.060	19.94	*0.086	*23.16	14.30
Total	0.320	106.21	*100.00

* By difference.

TABLE VII

A SUMMARY OF THE DATA ON FOREST-COVERED LOESS

Total N in Soil	0.128%	C/N	
Total Organic C in Soil	1.638%		
Carbonate CO	0.120%	12.79	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.0040	3.12
AB (NaOH)	0.0920	71.88	1.018	62.13	11.06
AB (NH ₄ OH)	0.821	50.12	1.795	0.45	45.73
B (NH ₄ OH)	0.570	34.80	1.160	0.25	49.14
B (NaOH)	0.0840	65.49	0.796	48.60	9.50
BA (HCl)
BAB (NaOH)	0.0140	10.93	0.310	18.92	22.15
BAB + H ₂ O	0.0075	5.86	0.234	14.29	31.20
Soil Residue	0.0235	18.36	*0.298	*18.20	12.68
Total	0.1290	100.64	*100.00

* By difference.

TABLE VIII

A SUMMARY OF THE DATA ON BERKELEY ADOBE

Total N in Soil	0.239%	C/N	
Total Organic C in Soil	2.919%		
Carbonate CO ₂	0.230%	12.21	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.010	4.18
AB (NaOH)	0.159	66.53	1.654	56.67	10.40
AB (NH ₄ OH)	1.260	43.17	2.39	0.48	52.72
B (NH ₄ OH)	0.658	22.54	1.24	0.33	53.07
B (NaOH)	0.157	65.69	1.385	47.45	8.82
BA (HCl)	0.007	2.93
BAB (NaOH)	0.026	10.87	0.505	17.30	19.42
BAB + H ₂ O	0.013	5.44	0.305	10.45	23.46
Soil Residue	0.034	14.22	*0.724	*24.80	21.29
Total	0.237	99.15	*100.00

* By difference.

TABLE IX
A SUMMARY OF THE DATA ON HEMPSTEAD SILT LOAM

Total N in Soil	0.256%	C/N
Total Organic C in Soil	3.373%	
Carbonate CO ₂	0.020%	13.17

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.008	3.12
AB (NaOH)	0.186	72.66	1.936	57.40	10.41
AB (NH ₄ OH)	1.865	55.30	3.61	0.52	51.66
B (NH ₄ OH)	0.929	27.54	2.00	0.62	46.45
B (NaOH)	0.168	65.63	1.530	45.36	9.10
BA (HCl)
BAB (NaOH)	0.039	15.24	0.730	21.64	18.72
BAB + H ₂ O	0.031	12.12	0.724	21.47	23.35
Soil Residue	0.033	12.89	*0.389	*11.53	11.79
Total	0.271	105.88	*100.00

* By difference.

TABLE X
A SUMMARY OF THE DATA ON MARSHALL SILT LOAM

Total N in Soil	0.237%	C/N
Total Organic C in Soil	2.670%	
Carbonate CO ₂	0.220%	11.27

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.009	3.80
AB (NaOH)	0.153	65.31	1.560	58.42	10.20
AB (NH ₄ OH)	1.510	56.56	2.85	0.37	52.99
B (NH ₄ OH)	0.791	29.63	1.49	0.39	53.09
B (NaOH)	0.152	64.13	1.270	47.56	8.36
B + B (NaOH)	0.048	20.25	0.470	17.60	9.79
(B + B) AB	0.019	8.02	0.377	14.12	19.84
(B + B) AB + H ₂ O	0.017	7.17	0.426	15.95	25.06
Soil Residue	0.025	10.55	*0.127	*4.76	5.08
Total	0.261	110.12	*100.00

* By difference.

TABLE XI
A SUMMARY OF THE DATA ON AN ACID "MUCK" SOIL

Total N in Soil	1.340%	C/N
Total Organic C in Soil	14.580%	
Carbonate CO ₂	None	10.88

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.034	2.54
AB (NaOH)	0.818	61.05	6.308	43.25	7.71
AB (NH ₄ OH)	3.715	25.48	7.14	0.30	52.04
B (NH ₄ OH)	2.814	19.30	5.82	11.88	48.35
B (NaOH)	0.778	58.06	5.374	36.85	6.91
BA (HCl)
BAB (NaOH)	0.151	11.27	1.798	12.33	11.91
BAB + H ₂ O	0.089	6.64	1.415	9.70	15.90
Soil Residue	0.303	22.61	*5.993	*41.10	19.78
Total	1.321	98.58	*100.00

* By difference.

† The water from this muck bog carried 0.2220 gm. of ash per liter. This ash was largely calcium sulphate.

TABLE XII
A SUMMARY OF THE DATA ON AN ACID SPHAGNUM-COVERED PEAT

Total N in Peat	2.00%	C/N
Total Organic C in Peat	49.32%	
Carbonate CO ₂	None	24.66

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.150	7.50	17.84
AB (NaOH)	1.468	73.41	26.20	53.13
AB (NH ₄ OH)	16.96	34.40	32.91	1.47	51.54
B (NH ₄ OH)	17.78	36.06	34.40	1.79	51.69
B (NaOH)	1.697	84.86	27.40	55.56	16.14
BA (HCl)
BAB (NaOH)	0.145	7.25	4.425	8.97	30.52
BAB + H ₂ O	0.066	3.30	2.06	4.18	31.22
Soil Residue	0.206	10.30	*15.43	*31.28	74.89
Total	2.114	105.71	*100.00

* By difference.

TABLE XIII
A SUMMARY OF THE DATA ON A CALCAREOUS BLACK GRASS-PEAT

Total N in Peat	2.94%	C/N
Total Organic C in Peat	42.81%	
Carbonate CO ₂	0.60%	14.56

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.081	2.76
AB (NaOH)	2.350	79.95	27.04	63.17	11.50
AB (NH ₄ OH)	14.70	34.34	28.71	1.74	51.19
B (NH ₄ OH)	6.51	15.21	12.97	1.13	50.19
B (NaOH)	2.146	73.00	22.68	52.98	10.57
BA (HCl)
BAB (NaOH)	0.390	13.26	7.42	17.34	19.02
BAB + H ₂ O	0.186	6.33	4.30	10.05	23.12
Soil Residue	0.119	4.05	*8.41	*19.64	70.68
Total	2.841	96.64	*100.00

* By difference.

TABLE XIV
A SUMMARY OF THE DATA ON AN ACID BROWN GRASS-PEAT

Total N in Peat	2.818%	C/N
Total Organic C in Peat	46.34%	
Carbonate CO ₂	None	16.44

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.066	2.34	12.90
AB (NaOH)	2.176	77.23	28.08	60.59	51.35
AB (NH ₄ OH)	20.14	43.45	39.22	1.87	49.19
B (NH ₄ OH)	17.36	37.45	35.29	2.25
B (NaOH)	2.143	76.05	27.19	58.66	12.69
BA (HCl)	0.041	1.46
BAB (NaOH)	0.281	9.97	4.88	10.53	17.37
BAB + H ₂ O	0.136	4.83	2.52	5.44	18.56
Soil Residue	0.202	7.17	*11.75	*25.36	58.17
Total	2.803	99.48	*100.00

* By difference.

TABLE XV

A SUMMARY OF THE DATA ON GRASS CUT FROM THE ACID BROWN PEAT BOG

Total N in Material	1.164%	C/N
Total Organic C in Material	47.020%	40.39

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	0.149	12.80	28.38
AB (NaOH)	0.948	81.43	26.900	57.22
AB (NH ₄ OH)	16.530	35.16	31.95	2.44	51.74
B (NH ₄ OH)	18.300	38.92	36.46	14.45	50.20
B (NaOH)	1.041	89.41	30.150	64.12	28.97
BA (HCl)	0.033	2.83
BA (NaOH)	0.037	3.18	2.370	5.04	64.00
BAB (NaOH)	None	None	0.451	0.96
BAB + H ₂ O	2.15	*14.050	*29.87	561.00
Soil Residue	0.025
Total	1.136	97.57	*100.00

* By difference.

† Probably largely inorganic plant constituents.

TABLE XVI

A SUMMARY OF THE DATA ON THE ENTIRE OAT PLANT

Total N in Material	2.131%	C/N
Total Organic C in Material	42.480%	39.48

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	0.737	34.58	16.43
AB (NaOH)	1.238	58.09	20.330	47.85
AB (NH ₄ OH)	14.400	33.90	26.65	2.63	54.04
B (NH ₄ OH)	17.150	40.36	36.09	14.62	47.51
B (NaOH)	1.976	92.72	26.470	62.30	13.40
BA (HCl)	0.019	0.89
BA (NaOH)	0.033	1.55	1.700	4.00	51.50
BAB (NaOH)	0.010	0.47	0.987	2.32	99.00
BAB + H ₂ O	0.049	2.30	*13.320	*31.35	368.00
Soil Residue	2.087	97.93	*100.00
Total

* By difference.

† Probably largely inorganic plant constituents.

TABLE XVII
A SUMMARY OF THE DATA ON ALFALFA HAY

Total N in Material	3.798%	C/N
Total Organic C in Material	44.260%	11.65

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	1.064	28.02
AB (NaOH)	2.500	65.83	16.51	37.31	6.61
AB (NH ₄ OH)	14.73	33.29	29.40	0.84	50.10
B (NH ₄ OH)	24.87	56.19	51.73	16.06	48.07
B (NaOH)	3.588	94.50	31.10	70.28	8.67
BA (HCl)	0.052	1.37
BAB (NaOH)	0.053	1.40	2.40	5.42	45.30
BAB + H ₂ O	0.008	0.21	2.01	4.54	251.00
Soil Residue	0.028	0.74	*8.75	*19.77	312.00
Total	3.729	98.22	*100.00

* By difference.

† Probably largely inorganic plant constituents.

TABLE XVIII
A SUMMARY OF THE DATA ON SWEET FERN LEAVES AND ON OAK LEAVES

	Sweet Fern Leaves	Oak Leaves
Total N in Material	1.585	0.998
Total Organic C in Material	48.340	48.630
C/N	30.500	48.700

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C			
Sweet Fern Leaves:							
A (HCl)	0.103	6.50
AB (NH ₄ OH)	15.64	32.35	31.81	3.20	49.17
B (NH ₄ OH)	21.54	44.55	41.70	2.46	51.65
Oak Leaves:							
A (HCl)	0.186	18.64
AB (NH ₄ OH)	14.83	30.50	29.81	2.34	49.76
B (NH ₄ OH)	17.51	35.99	35.94	2.23	48.71

TABLE XIX

A SUMMARY OF THE CARBON AND NITROGEN DATA IN TABLES III TO XVIII, SHOWING THE AVERAGE PERCENTAGES OF THE TOTAL NITROGEN AND TOTAL CARBON EXTRACTED BY THE DIFFERENT TREATMENTS IN SOILS, PEATS AND VEGETABLE MATERIALS

	A (HCl)	B (NH ₄ OH)	AB (NH ₄ OH)	AB (NaOH)	B (NaOH)	BAB (NaOH)	BAB + H ₂ O	Residue
Average per cent of Total N Extracted..								
8 Soils	3.17	68.53	64.69	14.11	8.34	14.91
4 Peats	3.78	72.91	72.99	10.44	5.28	11.03
3 ¹ and 5 ¹ Vegetable Materials	20.10	68.45	92.21	2.04	0.23	1.73
Average per cent of Total C Extracted..								
8 Soils	24.91	51.27	58.52	45.30	18.97	15.26	20.47
4 Peats	27.00	34.42	55.03	51.01	12.29	7.34	29.34
3 ¹ and 5 ¹ Vegetable Materials	43.20	33.40	47.46	65.57	4.82	2.61	26.99

¹ Sweet Fern Leaves and Oak Leaves were not extracted by any of the NaOH treatments, but the results from these materials appear in A (HCl), AB (NH₄OH) and B (NH₄OH).

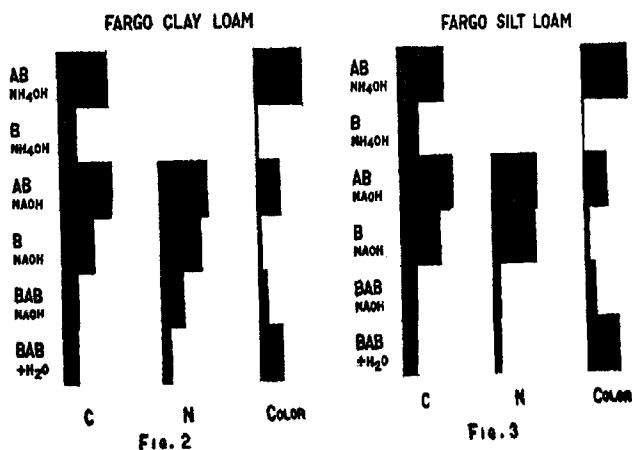


Fig. 2.—A graphic representation of the data on Fargo clay loam as given in Tables III and XXI.

Fig. 3.—A graphic representation of the data on Fargo silt loam as given in Tables IV and XXI.

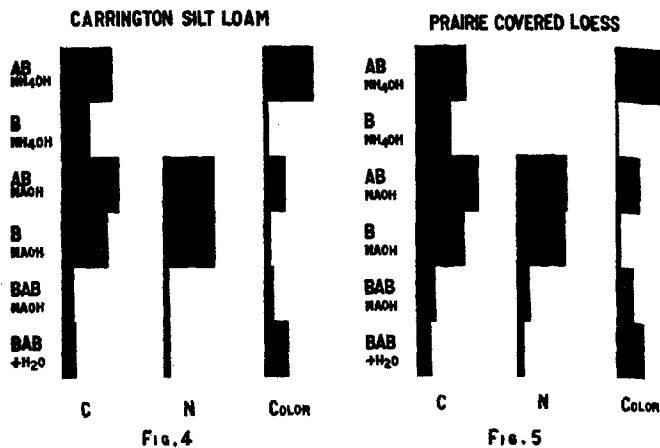


Fig. 4.—A graphic representation of the data on Carrington silt loam as given in Tables V and XXI.

Fig. 5.—A graphic representation of the data on prairie-covered loess as given in Tables VI and XXI.

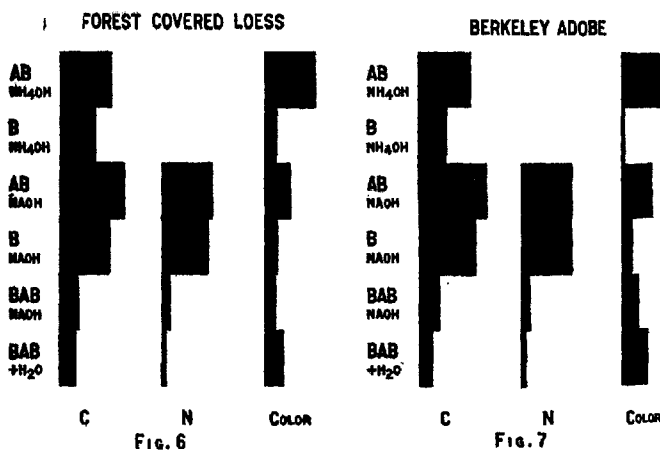


Fig. 6.—A graphic representation of the data on forest-covered loess as given in Tables VII and XXI.

Fig. 7.—A graphic representation of the data on Berkeley adobe as given in Tables VIII and XXI.

DISCUSSION

I. *Is the humus extract of soils a typical soil product, formed in the soil by the action of bacteria and fungi, or can a similar extract be obtained from unchanged vegetable material?* From a study of the data which have been presented in the preceding tables it would seem that only one conclusion is possible, i. e., the ordinary humus solution, AB (NH_4OH), contains to a very great extent dissolved substances which do not differ greatly in either color, carbon content, or nitrogen content

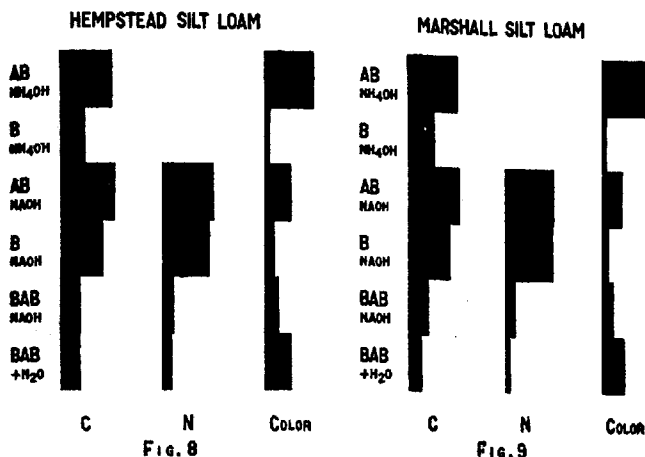


Fig. 8.—A graphic representation of the data on Hempstead silt loam as given in Tables IX and XXI.

Fig. 9.—A graphic representation of the data on Marshall silt loam as given in Tables X and XXI.

from similar substances which can be extracted by the same solvent from unchanged vegetable material. Table XXVI shows that a very considerable part of the ordinary humus is composed in reality of relatively colorless compounds. This fact is shown excellently by a reference to figures 2 to 10 in which carbon and nitrogen are compared with color. The carbon of B (NH_4OH) is undoubtedly largely present in AB (NH_4OH), only a small amount of carbon being soluble in the 1 per cent HCl .¹

¹In unpublished work the C/N ratio of the HCl extract was found to be approximately 14. The average percentage of nitrogen extracted from the eight soils by 1 per cent HCl was 0.00975 per cent of the soil, so that the average percentage of carbon extracted would be 0.140 per cent, or only 3.55 per cent of the (average) total carbon.

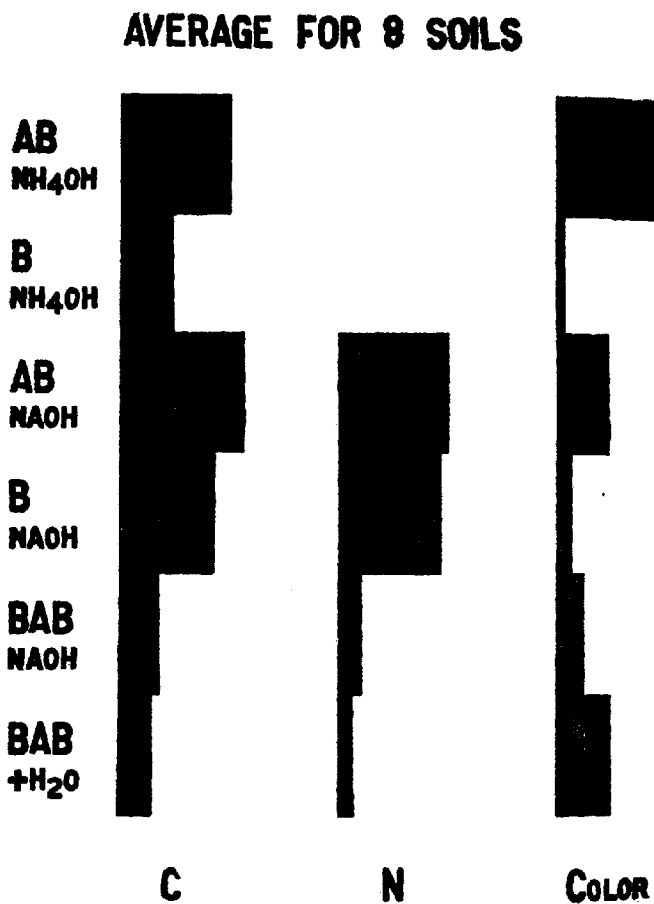


Fig. 10.—A graphic representation of the average data for the eight soils as given in Tables XIX and XXI.

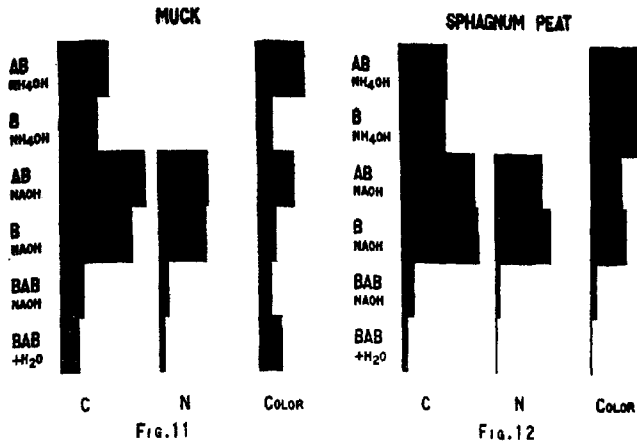


Fig. 11.—A graphic representation of the data on the acid muck soil as given in Tables XI and XXI.

Fig. 12.—A graphic representation of the data on sphagnum-covered peat as given in Tables XII and XXI.

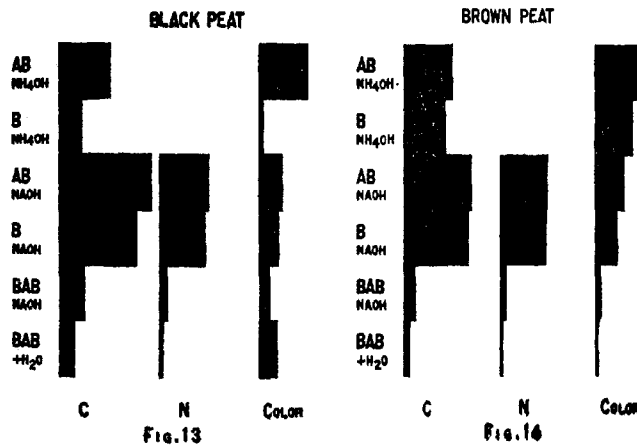


Fig. 13.—A graphic representation of the data on calcareous black peat as given in Tables XIII and XXI.

Fig. 14.—A graphic representation of the data on acid brown peat as given in Tables XIV and XXI.

Whereas, however, the carbon shows an average increase of about 113 per cent from $B(NH_4OH)$ to $AB(NH_4OH)$ the color increases nearly 1400 per cent, and because of this the presence of a large proportion of colorless organic compounds has not been generally recognized.

The ordinary humus solution $AB(NH_4OH)$ does contain the soil pigment, but as this is the only substance which appears with certainty to be a true soil product, and since the soil pigment probably rarely exceeds 35 to 40 per cent of the "humus," a determination of "humus" as ordinarily carried out, appears to be wholly without scientific justification.

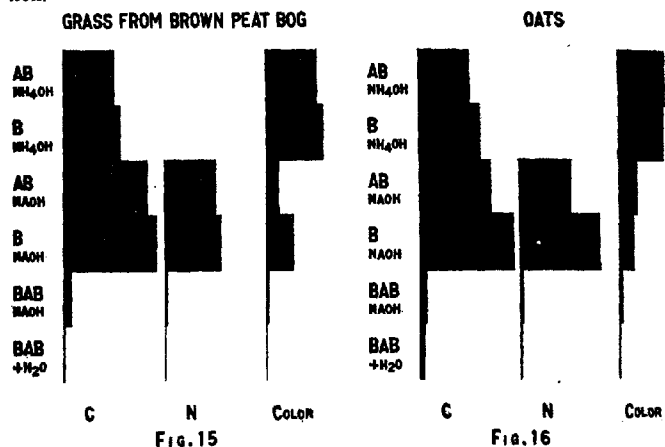


Fig. 15.—A graphic representation of the data on grass from brown peat bog as given in Tables XV and XXI.

Fig. 16.—A graphic representation of the data on the ripe oat plant as given in Tables XVI and XXI.

By no means all of the color of a "humus" solution is referable to the soil pigment, $BAB + H_2O$. The soil pigment gives a slaty black solution when viewed in a colorimeter and this black color cannot be used to match accurately the color of the $NaOH$ solutions which do not dissolve the black pigment but which do dissolve a red-brown color. This deep red-brown color is not peculiar to soil organic matter but can be obtained in equal concentration from *dry, dead, oak leaves, which have never touched the ground.*

Another notable example is that afforded by the sphagnum-covered peat. "Humic acids" have been prepared from sphagnum peats for years and yet the range of color, carbon and nitrogen in the sphagnum-covered peat extracts differs in no essential detail from that in the

unchanged vegetable materials. It seems to be impossible to reach any other conclusion but that this sphagnum-covered peat is, in all essential chemical relationships, a practically unchanged vegetable product, the only notable change which has taken place being a leaching out, or the decomposing, of a part of the easily soluble nitrogenous constituents [A (HCl) extract].

TABLE XXI

THE RELATIVE INTENSITY OF COLORATION OF THE SOLUTIONS WITHIN EACH SERIES AS MEASURED IN A COLORIMETER, AB (NH₄OH) BEING TAKEN AS UNITY

Soil or Vegetable Material Extracted	Treatment					
	AB(NH ₄ OH)	B(NH ₄ OH)	AB(NaOH)	B(NaOH)	BAB(NaOH)	BAB + H ₂ O
Fargo Silt Loam	1.00	20.00	2.00	9.60	4.16	1.47
Fargo Clay Loam	1.00	23.80	2.04	11.90	5.26	2.08
Forest-covered Loess	1.00	4.23	2.00	4.16	4.63	2.84
Prairie-covered Loess	1.00	20.00	2.00	10.70	3.03	1.70
Berkeley Adobe	1.00	9.43	1.51	4.31	2.73	1.81
Hempstead Silt Loam	1.00	9.43	1.92	5.35	3.70	1.96
Carrington Silt Loam	1.00	10.60	2.38	7.31	5.55	2.13
Marshall Silt Loam	1.00	13.80	2.50	8.06	4.41	2.17
Black Peat	1.00	10.80	2.08	2.50	4.54	2.77
"Muck"	1.00	3.24	1.35	2.72	3.78	2.12
Sphagnum-covered Peat	1.00	0.90	1.53	1.38	7.80	25.00
Brown Peat	1.00	1.25	1.56	2.13	10.00	20.00
Grass from Brown Peat Bog ..	1.00	0.91	4.31	1.96	26.30	colorless
Oats	1.00	1.03	2.63	3.33	17.00	colorless
Alfalfa Hay	1.00	0.82	1.43	2.50	colorless	colorless
Average for Soils (1-8)	1.00	13.91	2.04	7.67	4.18	2.02
Average for Peats (9-12)	1.00	4.05	1.63	2.18	6.53	12.49
Average for Vegetable Ma- terials (13-15)	1.00	0.92	2.79	2.60

(The figures represent the number of cubic centimeters of solution required to produce the same intensity of color as that possessed by 1 c.c. AB (NH₄OH))

The "humus" from the sphagnum-covered peat, from the brown peat, as well as that from the unchanged vegetable materials shows no true black, but only the red-brown color which when in concentrated solution is so dark as to appear black. Alway and Pinckney (4) have observed that colorimetric methods for humus are reliable only when the standard humus solution used is prepared from a soil of the same locality, or soil type, as are the humus solutions which are to be measured. This is now easily explained because we have at least *three* variables which influence the color of the humus solution, AB(NH₄OH), containing a unit weight of humus; (1) a black pigment, peculiar to the soil, (2)

a red-brown color extracted from dead plant materials, probably in an unchanged form and (3) a very considerable amount of practically colorless compounds. In addition to these we have still another phenomenon, *i. e.*,

TABLE XXII

THE INTENSITY OF COLOR IN THE ORDINARY HUMUS SOLUTIONS, AB (NH₄OH), AS COMPARED BOTH COLORIMETRICALLY AND PHOTOMETRICALLY WITH A 0.0063 PER CENT SOLUTION OF THE (ASH FREE) "SOIL PIGMENT"; ALSO COMPARISONS OF THE AMOUNT OF SOIL PIGMENT WITH THE TOTAL ORGANIC MATERIAL PRESENT IN THE SOLUTIONS

Soil Solution Taken	Color Equals mg. "Soil Pigment" per c.c.			Mg. "Humus" per c.c. Actually Found (gravimetric)	Indicated Approximate Percentage of Soil Pigment in the "Humus Solution"	Actual Percent of Carbon in the Humus Belonging to the Soil Pigment AB (C ₂ H ₄ O ₂) Carbon BAB + H ₂ O Carbon
	Colorimetric	Photometric	Average			
AB (NH ₄ OH)						
Fargo Silt Loam	0.60	0.46	0.53	1.69	31	29.5
Fargo Clay Loam	0.22	0.14	0.18	0.51	35	30.0
Forest-covered Loess	0.11	0.08	0.10	0.35	28	28.5
Prairie-covered Loess	0.19	0.16	0.18	0.63	28	28.8
Berkeley Adobe	0.13	0.09	0.11	0.46	24	24.2
Hempstead Silt Loam	0.26	0.23	0.25	0.71	35	38.8
Carrington Silt Loam	0.35	0.28	0.32	0.93	34	29.2
Marshall Silt Loam	0.22	0.16	0.19	0.55	34	27.5
Black Peat	0.41	0.36	0.39	1.79	22	29.2
"Muck"	0.11	0.08	0.10	0.62	16	38.0
Sphagnum-covered Peat	*	0.15	2.06	None	None
Brown Peat	*	0.13	2.32	None	None
Grass from Brown Peat Bog	*	0.06	1.95	None	None
Oats	*	0.029†	1.66	None	None
Alfalfa	*	0.008†	1.84	None	None
Sweet Fern Leaves	*	0.07	2.09	None	None
Oak Leaves	*	0.12	1.91	None	None
B (NH ₄ OH)						
Sweet Fern Leaves	*	0.16	2.74	None	None
Oak Leaves	*	0.08	2.35	None	None

* It was impossible to read these solutions in a colorimeter against the soil pigment solution, inasmuch as they contained no black color, being merely more or less intense red brown.

† Not dark enough for photometric readings, values interpolated by comparing them colorimetrically with a sphagnum-covered peat AB (NH₄OH) standard.

colors produced by plant indicators. A large part of the color in the ammonia extract from the grass from the brown peat bog appears to be due to the presence of the hydroxyl ion. The hydrochloric acid extract of this grass was of a light straw color but the addition of ammonia caused

TABLE XXIII
RELATIVE AMOUNTS OF ORGANIC CARBON IN EACH SOLUTION IN EACH SERIES,
AB (NH₄OH) BEING TAKEN AS UNITY

Soil or Vegetable Material Taken	Treatment					
	AB(NH ₄ OH)	B(NH ₄ OH)	AB(NaOH)	B(NaOH)	BAB(NaOH)	BAB+H ₂ O
Fargo Silt Loam	1.00	2.25	0.86	1.13	3.14	3.16
Fargo Clay Loam	1.00	3.04	0.94	1.49	2.69	3.33
Forest-covered Loess	1.00	1.43	0.80	1.02	2.66	3.48
Prairie-covered Loess	1.00	2.46	0.84	1.12	2.16	3.48
Berkeley Adobe	1.00	1.92	0.76	0.91	2.51	4.10
Carrington Silt Loam	1.00	1.77	0.88	1.09	3.91	3.46
Hempstead Silt Loam	1.00	2.04	0.95	1.21	2.54	2.57
Marshall Silt Loam	1.00	1.89	0.96	1.17	2.51	3.65
Black Peat	1.00	2.26	0.54	0.65	1.99	3.40
"Muck"	1.00	1.31	0.58	0.69	2.06	2.61
Sphagnum-covered Peat	1.00	0.95	0.63	0.61	3.82	8.31
Brown Peat	1.00	1.16	0.71	0.74	4.13	8.04
Grass from Brown Peat Bog	1.00	0.90	0.61	0.55	7.00	36.00
Oats	1.00	0.83	0.71	0.54	8.47	14.50
Alfalfa	1.00	0.48	0.89	0.47	6.14	7.30
Average for Soils (1-8)....	1.00	2.10	0.89	1.14	2.77	3.40
Average for Peats (9-12)....	1.00	1.42	0.61	0.67	3.60	5.59
Average for Vegetable Materials (13-15)	1.00	0.74	0.74	0.52	7.20	19.26

(The figures represent the number of cubic centimeters of solution necessary to contain the same weight of organic carbon that is contained in 1 c.c. AB (NH₄OH))

TABLE XXIV
HUMUS AND HUMUS CARBON (FOUND AND CALCULATED) ON UNLEACHED SOILS

	Humus Per cent	Humus Carbon			
		Per cent Calc. ¹	Per cent Found	Error	Per cent Error
Marshall Silt Loam	1.49	0.745	0.791	-0.046	-6.1
Fargo Clay Loam	0.95	0.475	0.476	-0.001	-0.2
Fargo Silt Loam	3.65	1.825	1.775	+0.050	+2.8
Carrington Silt Loam	2.86	1.430	1.436	-0.006	-0.4
Prairie-covered Loess	1.42	0.710	0.702	+0.008	+1.1
Forest-covered Loess	1.16	0.580	0.570	+0.010	+1.7
Hempstead Silt Loam	2.00	1.000	0.929	+0.071	+7.6
Berkeley Adobe	1.24	0.621	0.658	-0.037	-5.6
Sphagnum-covered Peat	34.40	17.200	17.780	-0.580	-3.2
Black Peat	12.97	6.490	6.510	-0.020	-3.0
Brown Peat	35.29	17.640	17.360	+0.280	+1.6
"Muck"	5.82	2.910	2.814	+0.096	+3.4
Alfalfa	51.73	25.860	24.870	+0.990	+3.9
Oats	36.09	18.040	17.150	+0.890	+5.1
Grass from Brown Peat Bog	36.46	18.230	18.300	-0.070	-3.8
Sweet Fern Leaves	41.70	20.850	21.540	-0.690	-3.2
Oak Leaves	35.94	17.970	17.510	+0.460	+2.6

¹ Calculated by halving the humus percentages (humus carbon = $\frac{\text{humus}}{2}$).

this solution to turn a quite dark red-brown which again became relatively colorless, without the formation of a precipitate, on the addition of acid. The hydrochloric acid extract of a soil cannot be tested in a similar manner because of the presence of iron and aluminium compounds which adsorb the indicator, but undoubtedly vegetable indicators are responsible for a part of the color in both ammonia and sodium hydroxide solutions.

All of the color data seem to indicate that, given the same materials ammonium hydroxide will dissolve *less* carbon and at the same time produce a *much darker solution* than will sodium hydroxide. Just why this

TABLE XXV
HUMUS AND HUMUS CARBON (FOUND AND CALCULATED) ON LEACHED SOILS

	Humus Per cent	Humus Carbon			
		Per cent Calc. ¹	Per cent Found	Error	Per cent Error
Marshall Silt Loam	2.850	1.425	1.510	-0.085	-5.6
Fargo Clay Loam	2.660	1.330	1.423	-0.093	-6.5
Fargo Silt Loam	9.910	4.955	5.174	-0.219	-4.2
Carrington Silt Loam	4.950	2.475	2.525	-0.050	-1.9
Prairie-covered Loess	3.400	1.700	1.737	-0.037	-2.1
Forest-covered Loess	1.795	0.897	0.821	+0.076	+9.2
Hempstead Silt Loam	3.610	1.805	1.865	-0.060	-3.2
Berkeley Adobe	2.390	1.195	1.260	-0.065	-5.1
Sphagnum-covered Peat	32.910	16.450	16.960	-0.510	-3.0
Black Peat	28.710	14.350	14.700	-0.350	-2.4
Brown Peat	39.220	19.610	20.140	-0.530	-2.6
"Muck"	7.140	3.570	3.715	-0.145	-3.9
Alfalfa	29.400	14.700	14.730	-0.030	-0.2
Oats	26.650	13.330	14.400	-0.930	-6.4
Grass from Brown Peat Bog ..	31.950	15.980	16.530	-0.550	-3.3
Sweet Fern Leaves	31.810	15.900	15.640	+0.260	+1.6
Oak Leaves	29.810	14.900	14.830	+0.070	+0.4

humus

¹ Calculated by halving the humus percentages (humus carbon = $\frac{\text{humus}}{2}$).

should be the case it is difficult to state, but the color data for the vegetable materials in Table XXI show that the color of the ammonia AB (NH_4OH) solutions was more than twice as intense as that of the corresponding NaOH solutions. Hilgard (17, p. 125-126, 132) refers to NaOH as the more efficient "humifier" presumably meaning that it produces color from organic compounds more readily than does an ammonia solution so that the answer apparently does not lie in a chemical change. It is possible that a 4 per cent concentration of the fixed alkali is great enough to shift a color equilibrium. This could be tested relatively easily by measuring the intensity of coloration at different dilutions but has not been attempted in this connection.

II. Does a 4 per cent sodium hydroxide solution extract the same substances from a soil as does a 4 per cent ammonium hydroxide solution, and if the same substances are extracted is the same quantity extracted by

each solvent? It is surprising that this question has not been answered long ago. Hilgard and Jaffa (18) originated a method for the determination of "humus nitrogen" in which the ammonia extraction of the "humus" was replaced by a sodium, or potassium hydroxide extraction, and nitrogen determined by Kjeldahling the resulting solution. Unless the same substances are extracted in the same amounts there is no justification for assuming that the nitrogen obtained by this process is a quantita-

TABLE XXVI
COMPARISON OF THE PERCENTAGES OF HUMUS OBTAINED FROM THE LEACHED SOIL AB (NH_4OH) WITH THOSE OBTAINED FROM THE UNLEACHED SOIL, B (NH_4OH) AND THE DIFFERENCE BETWEEN THESE VALUES WITH THE PERCENTAGES OF SOIL PIGMENT ($\text{BAB} + \text{H}_2\text{O CARBON} \times 2$)

	Humus B(NH_4OH) Per cent	Humus AB(NH_4OH) Per cent	Increase	Approximate Per cent of "Soil Pigment" in Soil (BAB + $\text{H}_2\text{O C.} \times 2$)	Difference Due to Relatively Colorless Compounds
Fargo Silt Loam	3.65	9.91	6.26	3.06	3.20
Fargo Clay Loam	0.95	2.66	1.71	0.86	0.85
Forest-covered Loess	1.16	1.80	0.64	0.47	0.17
Prairie-covered Loess	1.42	3.40	1.98	1.00	0.98
Berkeley Adobe	1.24	2.39	1.15	0.61	0.54
Hempstead Silt Loam	2.00	3.61	1.61	1.45	0.16
Carrington Silt Loam	2.86	4.95	2.19	1.47	0.72
Marshall Silt Loam	1.49	2.85	1.36	0.85	0.51
Average for Soils (1-8)	1.85	3.95	2.10	1.22	0.88
Black Peat	12.97	28.71	15.74	8.60	7.14
"Muck"	5.82	7.14	1.32	2.83
Sphagnum-covered Peat	34.40	32.91	-1.49	None
Brown Peat	35.29	39.22	3.93	None
Grass from Brown Peat Bog	36.46	31.95	-4.51	None
Oats	36.09	26.65	-9.44	None
Alfalfa Hay	51.73	29.40	-22.33	None
Sweet Fern Leaves	41.70	31.81	-9.89	None
Oak Leaves	35.94	29.81	-6.13	None

¹ It is highly improbable that this is all "soil pigment," for the solution measured colorimetrically gave only 0.75 per cent soil pigment.

tive (or even qualitative) measure of the nitrogen in the "humus" obtained in the other process. Tables XX, XXI, XXIII, XXVII and XXVIII as well as figures 2 to 10 and the four figures on Plates I and II, show that no direct relationship exists. To be sure the carbon contents of AB (NH_4OH) and AB (NaOH) do not differ widely, the range being +7.2 to +38.5 per cent of humus, in each instance in favor of the fixed alkali, but the color of the extracts averages more than 100 per cent greater in favor of the ammonia solution. As for the nitrogen content of the two solutions, it is impossible to make a definite statement.

Table XXX shows a comparison between a few nitrogen determinations run on the ammonia solutions and the corresponding sodium hydroxide extractions. Nitrogen was determined in the ammonia solution by evaporating 200 to 300 c.c. of the 4 per cent ammonia extract *in vacuo* at 45° to 50° C. after having first added about 10 c.c. of a 10 per cent $\text{Ca}(\text{OH})_2$ suspension and continuing the evaporation until the ammonia distilled off during 5 minutes was not sufficient to neutralize 4 drops of $\text{N}/14 \text{H}_2\text{SO}_4$. The apparatus used for the distillation is the same as is used by Van Slyke (31) for the determination of ammonia in the hydrolysate of

TABLE XXVII
DIFFERENCE BETWEEN THE AMOUNTS OF ORGANIC MATTER EXTRACTED FROM
THE UNLEACHED SOILS BY 4 PER CENT NaOH AND BY 4 PER CENT NH_4OH

Soil or Vegetable Material Extracted	Per cent Carbon $\text{B}(\text{NaOH})$	Per cent "Humus" $\text{B}(\text{NaOH})$ (Calc.) ¹	Per cent "Humus" found $\text{B}(\text{NH}_4\text{OH})$	Increase in favor of NaOH	Per cent Increase
Fargo Silt Loam	4.555	9.11	3.65	5.46	149
Fargo Clay Loam	0.979	1.96	0.95	1.01	106
Forest-covered Loess	0.796	1.59	1.16	0.43	37
Prairie-covered Loess	1.548	3.10	1.42	1.68	118
Berkeley Adobe	1.385	2.77	1.24	1.53	123
Hempstead Silt Loam	1.530	3.06	2.00	1.06	53
Carrington Silt Loam	2.349	4.70	2.86	1.84	64
Marshall Silt Loam	1.270	2.54	1.49	1.05	70
Black Peat	22.680	45.36	12.97	32.39	249
"Muck"	5.370	10.75	5.82	4.93	84
Sphagnum-covered Peat	27.400	54.80	34.40	20.40	59
Brown Peat	27.190	54.38	35.29	19.09	54
Grass from Brown Peat Bog ..	30.150	60.30	36.46	23.84	65
Oats	26.470	52.94	36.09	16.85	46
Alfalfa Hay	31.100	62.20	51.73	10.47	22
Average for Soils (1-8)	1.801	3.60	1.85	1.75	94
Average for Peats (9-12)	20.660	41.32	22.12	19.20	86
Average for Vegetable Materials (13-15)	29.240	58.48	41.43	17.05	41

¹ Calculated by multiplying the organic carbon by 2 (humus = humus carbon \times 2).

proteins. The residue remaining in the flask after the removal of the ammonia was Kjeldahled as usual. The data are too meager for any generalization but they seem to indicate that a method has been found which can be utilized to determine the soil nitrogen in ammonia extracts with a fair degree of accuracy. No relationship between NaOH nitrogen and NH_4OH nitrogen is indicated by the data available.

In this connection it is of interest to compare the results which were obtained on Berkeley adobe with those obtained by Alway and Bishop (1). As already indicated, the present sample of soil was prepared by mixing their samples "A" and "B." Unfortunately the samples were not weighed before mixing so that an accurate comparison is impossible. Using the

Rather method they obtained 1.71 per cent of humus for "A" and 1.19 per cent for "B." For the mixed soils is obtained by my methods 2.39 per cent, or considerably more than they obtained for their sample "A." They obtained 0.160 per cent and 0.119 per cent of "humus nitrogen" (by NaOH) while the writer obtained 0.159 per cent. Their "nitrogen in humus," obtained by using the AB (NaOH) figures for N and the AB (NH₄OH) figures for humus, were 9.3 per cent and 10.0 per cent. My value would be only 6.65 per cent.

TABLE XXVIII
DIFFERENCE BETWEEN THE AMOUNTS OF ORGANIC MATTER EXTRACTED FROM
THE LEACHED SOILS BY 4 PER CENT NaOH AND BY 4 PER CENT NH₄OH

Soil or Vegetable Material Extracted	Per cent Carbon AB(NaOH)	Per cent "Humus" AB(NaOH) (Calc.) ¹	Per cent "Humus" Found AB(NH ₄ OH)	Increase in Favor of NaOH	Per cent Increase
Fargo Silt Loam	5.990	11.980	9.910	2.070	20.9
Fargo Clay Loam	1.537	3.070	2.660	0.410	15.4
Forest-covered Loess	1.018	2.040	1.800	0.240	13.3
Prairie-covered Loess	2.049	4.100	3.400	0.700	20.6
Berkeley Adobe	1.654	3.310	2.390	0.920	38.5
Hempstead Silt Loam	1.936	3.870	3.610	0.260	7.2
Carrington Silt Loam	2.890	5.780	4.950	0.830	16.7
Marshall Silt Loam	1.560	3.120	2.850	0.270	9.4
Black Peat	27.040	54.080	28.710	25.370	88.3
"Muck"	6.308	12.620	7.140	5.480	76.7
Sphagnum-covered Peat	26.200	52.400	32.910	19.490	59.2
Brown Peat	28.080	56.160	39.220	16.940	43.2
Grass from Brown Peat Bog ...	26.900	53.800	31.950	21.850	68.3
Oats	20.330	40.660	26.650	14.010	52.6
Alfalfa Hay	16.510	33.020	29.400	3.620	12.3
Average for Soils (1-8)	2.329	4.658	3.946	0.712	17.9
Average for Peats (9-12)	21.910	43.820	26.990	16.820	62.3
Average for Vegetable Materials (13-15)	21.250	42.490	29.330	13.160	44.8

¹ Calculated by multiplying the organic carbon by 2 (humus = humus carbon × 2).

These authors question Hilgard's generalization that the humus of arid soils is richer in nitrogen than is the humus from humid regions. This finding is substantiated by a comparison of the C/N ratios for AB (NaOH). There is no false comparison possible here, as in the case of comparing ammonia humus with NaOH nitrogen figures, and a low C/N ratio should indicate a high percentage of nitrogen in the humus. Tables XXXII shows the C/N ratios for both the leached and unleached soils. The typical humid, bottom land soil, Fargo silt loam, shows the highest content of nitrogen in the humus, AB (NaOH). Three of the soils contain even more nitrogen in proportion to the total carbon in the AB (NaOH) solutions than does the Berkeley adobe, while in five of the eight soils the B (NaOH) humus is richer in nitrogen than is the adobe.

Of course, these data from a single sample are not conclusive proof that arid soils do not contain a humus which is richer in nitrogen than that obtained from soils of humid regions, but taken in connection with Alway and Bishop's (1) data it would seem to corroborate their conclusion that such a generalization is open to serious criticism.

TABLE XXIX
RELATIVE PERCENTAGES OF CARBON AND NITROGEN EXTRACTED FROM THE
UNLEACHED SOIL BY 2 PER CENT NaOH AND BY 4 PER CENT NaOH,
TOGETHER WITH C/N RATIOS

	Per cent Nitrogen			Per cent Carbon			C/N 2% NaOH	C/N 4% NaOH
	2% NaOH	4% NaOH	Increase	2% NaOH	4% NaOH	Increase		
Fargo Silt Loam	0.497	0.584	0.087	3.928	4.555	0.627	7.90	7.80
Fargo Clay Loam	0.102	0.127	0.025	0.787	0.979	0.192	7.71	7.71
Forest-covered Loess..	0.075	0.084	0.009	0.820	0.798	(-)0.024	10.93	9.50
Prairie-covered Loess..	0.166	0.186	0.020	1.374	1.548	0.174	8.18	8.32
Berkeley Adobe	0.141	0.157	0.016	1.268	1.385	0.117	8.99	8.82
Hempstead Silt Loam..	0.168	0.168	None	1.556	1.530	(-)0.026	9.26	9.10
Carrington Silt Loam..	0.246	0.271	0.025	2.274	2.349	0.075	9.24	8.67
Marshall Silt Loam...	0.145	0.152	0.007	1.114	1.270	0.156	7.68	8.36
Black Peat	1.863	2.146	0.283	18.980	22.680	3.700	10.18	10.57
Sphagnum-cove'd Peat	1.547	1.697	0.150	25.900	27.400	1.500	16.74	16.14
"Muck"	0.730	0.778	0.048	5.628	5.374	(-)0.254	7.71	6.91

TABLE XXX
PERCENTAGES OF SOIL NITROGEN EXTRACTED BY AMMONIUM HYDROXIDE
COMPARED WITH THE AMOUNTS EXTRACTED BY SODIUM HYDROXIDE

	B (NH ₄ OH)			B (NaOH)	AB (NH ₄ OH)			AB (NaOH)
	I	II	Av.		I	II	Av.	
Fargo Silt Loam	0.250	0.258	0.254	0.584	0.562	0.584	0.573	0.613
Marshall Silt Loam	0.194	0.194	0.153
Hempstead Silt Loam..	0.145	0.142	0.144	0.168
Carrington Silt Loam..	0.215	0.249	0.232	0.271
Prairie-covered Loess..	0.117	0.127	0.122	0.186
Black Peat	0.889	0.869	0.879	2.146

As regards the solubility of the coloring material, Alway and Bishop (1) recently made the following statement:

"We have confirmed Hilgard and Jaffa's observation that after prolonged extraction of a soil with either ammonia or alkaline hydroxide solution the other fails to extract any appreciable amount of black material."

Using the present methods the author is able to confirm their observation that, after prolonged extraction with ammonia, the 4 to 6 per cent sodium hydroxide solution fails to extract any appreciable amount of black material, but not the reverse. In every case the black pigment of

TABLE XXXI

RESULTS INDICATING THAT PERHAPS THE ORGANIC CARBON OF PEATS AND VEGETABLE MATERIALS CAN BE DETERMINED WITH A FAIRLY HIGH DEGREE OF ACCURACY BY DIVIDING THE VOLATILE MATTER BY AN EMPIRIC FACTOR

	Per cent "Volatile"	Per cent Carbon Calculated	Per cent Carbon Actually Found	Error	Per cent Error
Peats		Volatile			
		1.842			
Black Peat	78.68	42.71	42.81	(-)0.10	(-)0.23
Brown Peat	86.10	46.74	46.34	+0.40	+0.86
Sphagnum-covered Peat	90.59	49.17	49.32	-0.15	-0.30
"Muck"	26.75	14.52	14.58	-0.06	-0.04
Vegetable Materials		Volatile			
		1.971			
Alfalfa	88.47	44.88	44.26	+0.62	+1.40
Oats	86.29	43.78	42.51	+1.27	+2.98
Oak Leaves	94.13	47.75	48.63	-0.88	-1.80
Sweet Fern Leaves	92.44	46.90	48.34	-1.44	-2.97
Grass from Brown Peat Bog ...	92.97	47.17	47.02	+0.15	+0.32

TABLE XXXII

C/N RATIOS FOR THE LEACHED AND UNLEACHED SOIL EXTRACTS

	Fargo Clay Loam	Fargo Silt Loam	Carrington Silt Loam	Hemphstead Silt Loam	Prairie-covered Loess	Forest-covered Loess	Marshall Silt Loam	Berkeley Adobe
AB (NaOH)	10.05	9.77	10.86	10.41	10.56	11.06	10.20	10.40
B (NaOH)	7.71	7.80	8.67	9.10	8.32	9.50	8.36	8.82
BAB (NaOH)	7.59	18.30	21.77	18.72	16.29	22.15	19.83	19.42
BAB + H ₂ O	15.29	19.13	23.03	23.35	20.00	31.20	31.92	23.46

the soil was found to be *quite insoluble* in 4 per cent NaOH, so much so that the addition of NaOH up to a 4 per cent concentration to a solution of the soil pigment in dilute alkali (0.1 per cent NaOH) causes a precipitation of the pigment.

The method of testing the solubility of the residue in ammonia after extraction with sodium hydroxide used by Alway and Bishop, as de-

scribed to me by the former of these authors, and doubtless also that used by Hilgard and Jaffa, was the Hilgard-Jaffa method of extracting on a cone-shaped filter, with a protecting disk of filter paper, adding the extracting fluid in small portions until the filtrate became practically colorless (*cf.* Alway, Files, and Pinckney, [3] 1910).

Taking the Marshall silt loam, which was from the same field as the humid soil that Alway and Bishop used, it was leached on a filter, following a previous leaching with HCl, with 4 per cent NaOH until the solution came through colorless and then leached, without removing it from the filter, with 4 per cent NH_4OH . The first 100 c.c. were almost colorless and even the first day's leachings, totaling about 500 c.c., were not black as had been expected, but light brown. The second and third day's leachings, totaling about 300 c.c. were, however, intensely black, and further leachings rapidly became colorless. *Had the author not been certain that a soluble pigment remained in the soil residue after the NaOH treatment, he should probably have not leached with more than 50 to 100 c.c. of ammonia, and thus have arrived at the same conclusion as previous investigators.* It is strange that the pigment does not dissolve in the first portions of the ammonia poured through the filter. It may be that the deflocculated soil is at first impervious to the ammonia solution and that the filtrate obtained is simply what passes between the soil mass and the filter without penetrating the soil. The sodium hydroxide in the external part of the soil mass probably has for some time a sufficient concentration to prevent a solution of the soil pigment which dissolves in the presence of NaOH only when the concentration of the fixed alkali is very low.

When instead of extracting with ammonia on a funnel, the residue from the NaOH extraction was placed in a beaker, or cylinder, ammonia added and the soil mass thoroughly disintegrated, after which the clay was flocculated with ammonium carbonate, the solution was found to be intensely black.

These observations serve to explain the erroneous conclusions of Hilgard and Jaffa, of Alway and Bishop, and possibly of numerous other investigators who have not published their confirmation of Hilgard and Jaffa's observations.

The following experiments were made in order to test out quantitatively the author's observations as to a difference in solubility in the two solvents. Two portions of 15 gm. each, of the same soil type (Fargo silt loam), but differing in that one contained only 0.20 per cent of carbonate CO_2 , while the other contained 9.90 per cent were leached with 1 per cent HCl, and extracted in the shaking machine three successive periods of 4 days each with fresh quantities of 750 c.c. 4 per cent NH_4OH . "Humus" was determined on each extract. Similar extractions were made, with the use of successive portions of 4 per cent NaOH. At the end of the three

extractions the contents of the bottles were acidified with HCl, the precipitated soil washed first by decantation and later on a filter until free from chlorides, and then 4 per cent NaOH was added to the residue from the NH_4OH treatment, and NH_4OH to the residue from the NaOH extractions. The final portion of sodium hydroxide did not dissolve any black color but the solution became a quite intense red-brown while the final ammonium hydroxide solution was *jet black*. Table XXXIII and figure 17 show the amount of

TABLE XXXIII

AMOUNTS OF CARBON, "HUMUS" AND COLOR OBTAINED FROM TWO SOILS IN THREE SUCCESSIVE EXTRACTIONS WITH 4 PER CENT NH_4OH , FOLLOWED BY A SINGLE EXTRACTION WITH 4 PER CENT NaOH, AS WELL AS THE REVERSE SERIES OF THREE EXTRACTIONS WITH 4 PER CENT NaOH FOLLOWED BY A SINGLE EXTRACTION WITH 4 PER CENT NH_4OH

	Soil 1. Organic C. 10.02% Carbonate CO_2 0.20%				Soil 2. Organic C. 10.03% Carbonate CO_2 9.90%			
	Per cent Carbon	Per cent Humus	Per cent Humus Ash	Color ¹	Per cent Carbon	Per cent Humus	Per cent Humus Ash	Color ¹
1st extr. NaOH.....	5.58	350	4.99	340
2nd extr. NaOH.....	0.71	33	0.80	42
3rd extr. NaOH.....	0.26	14	0.32	15
Residue + NH_4OH ...	*1.16	2.33	0.63	467	*1.40	2.80	0.37	540
1st extr. NH_4OH	2.60	5.21	0.41	966	3.16	6.33	*1.16	1066
2nd extr. NH_4OH	0.69	1.37	0.42	62	0.72	1.44	1.09	76
3rd extr. NH_4OH	0.37	0.74	0.23	32	0.39	0.78	0.86	30
Residue + NaOH....	1.61	63	1.88	63

¹ Color is referred to a 0.0063 per cent solution of the ash-free soil pigment, a ratio of 100 equalling the color of this pigment solution, while a ratio of 1000 would equal the color of a 0.063 per cent solution.

* The carbon figures for NH_4OH solutions are obtained by dividing the humus by 2 (cf. Tables XXIV and XXV).

² The high "humus ash" figures in this column do not seem to be due to suspended clay but to dissolved mineral matter. This is the only soil I have found which always gives a high humus ash. The solubility continues practically the same through three successive extractions with NH_4OH .

carbon, "humus" and color actually obtained on the different solutions. It can be readily seen that these data agree in all respects with the conclusions indicated by the preceding tables and discussions, *i. e.*, *Sodium hydroxide does not dissolve either the same substances or the same quantity of substances from a soil as does ammonium hydroxide.*

III. *Is the soil nitrogen present in a different form from that in which the nitrogen occurs in plant materials, or does the nitrogen of the soil exhibit the same solubilities as vegetable nitrogen?*

From a study of the preceding tables it becomes evident that the nitrogen of the soil is not exactly similar to that of unchanged vegetable ma-

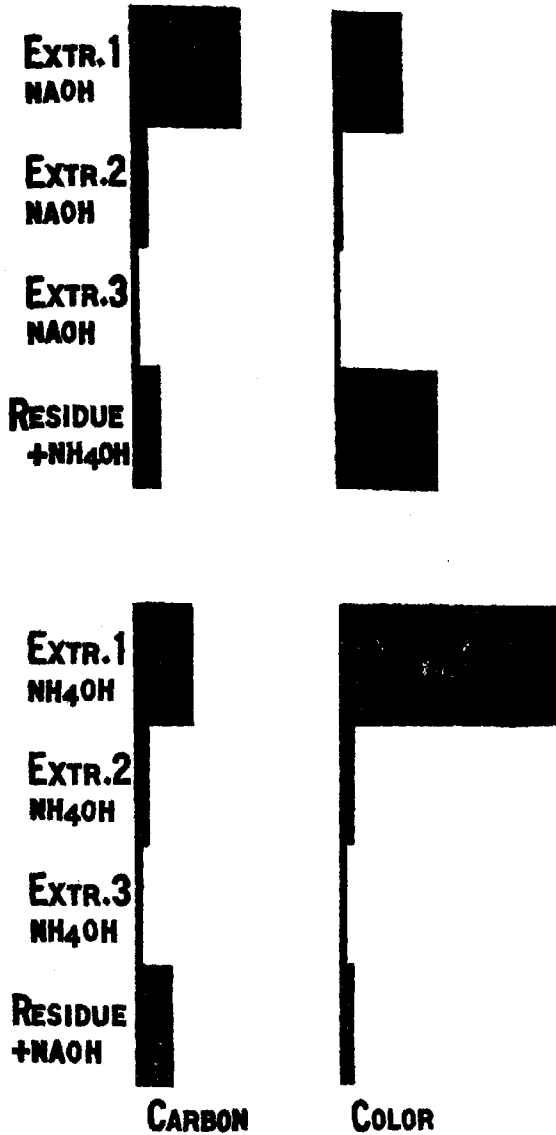


Fig. 17.—A graphic representation of the average of the determinations given in Table XXXIII.

terials. Summary Table XIX shows the comparison. The principal difference seems to be that the easily soluble forms of nitrogen, such as dissolve in 1 per cent HCl, do not long remain in the soil, while, on the other hand, there is a considerable amount of nitrogen in the soil which exhibits marked insolubility in NaOH solutions, in decided contrast to the behavior of the vegetable materials tested. It seems possible that the insoluble nitrogen may represent, at least in part, the nitrogen of the spores of bacteria and fungi. The easily soluble nitrogen is apparently very readily broken down in the soil to ammonia and nitrates, or possibly it may leach out.

It is of interest to note that no very large portion of the soil nitrogen is rendered insoluble by the lime and magnesia of the soil. Potter and Snyder (24) call attention to the necessity of leaching soils in order to set free the proteins from their calcium salts and thus render the proteins soluble. A reference to Table XIX shows that, for the average of the eight soils, leaching increased the amount of nitrogen dissolved less than 4 per cent, the color increasing, however (Table XXI), 376 per cent. In other words, the substances which impart color to the soil contain only a small percentage of nitrogen, the average ratio of C/N in the soil pigment (BAB+H₂O) being 22.6 for the eight soils or, assuming that the materials contain 50 per cent of carbon, a nitrogen content of only 2.21 per cent. It thus appears to be a coincidence that the darker soils from the same locality are, in general, the more fertile.

It will be noted that two of the acid peats contain none of the soil pigment while the "acid muck" contains but very little. These soils present very unfavorable media for bacterial growth; indeed, it has long been recognized that acid peat bogs are practically free from bacteria at no great distance from the surface. On the other hand the calcareous peat contains a large amount of the black pigment.

Beijerinck (7) found that *Azotobacter chroococcum* excretes a dark brown or black pigment and Hall (15, p. 188) suggests that this pigment may play its part in the coloration of humus. It may well be that BAB + H₂O, the soil pigment, is produced by a beneficial bacterium. Its presence and amount would accordingly be an indication of the bacterial activity and thus the quantity present would be roughly proportional to the soil fertility. Unfortunately, it was impossible to secure bacteriological data on the soils used, but it is to be hoped that some investigator will attempt to answer the problem: *Is the black pigment a bacterial product?*¹

¹ Since the manuscript of this paper was completed a paper has appeared by Briggs, Jensen and McLane (8), in which they present data showing that there is a high degree of correlation between the "humus" content of the soil and "mottle-leaf" of citrus trees. Their humus percentages were determined colorimetrically against a standard humus solution.

Alway and Pinckney (4) and Alway and Blish (2) have shown that the colorimetric method for humus is approximately accurate only when the standard humus solution is prepared from a soil of the same soil type and locality as the sample under investigation. Kelly and McGeorge (19,

IV. *The Relationship of carbon to humus.* After all of the humus carbon determinations had been made it was found that humus carbon can be determined within experimental error by halving the humus figures. This is shown in Tables XXIV and XXV. It seems probable that the carbon content of the "unleached humus" is slightly lower than 50 per cent while that of the "leached humus" is slightly above 50 per cent, but the slight differences are in practically all cases within experimental error. The fact that the same factor applies to the ammonia extracts from peats and unchanged vegetable materials, speaks very strongly in support of the contention that the soil organic matter does not differ to any marked extent from that of unchanged plant materials and that a specific "humification," giving rise to a *humus* in which the nitrogen is more readily available for plant-food than in unchanged vegetable materials does not take place.

p. 18), dealing with Hawaiian soils, mention a series of "humus" solutions which were of a yellowish straw color" but which gave a gravimetric humus percentage exceeding 3 per cent. Alway and Pinckney (4, p. 4) state that they could not obtain satisfactory results when the colorimetric method was applied to a number of desert soils.

As the author has shown earlier in this paper, there are four variables which influence the color of the solution containing a unit weight of humus, *i. e.*, the amount of black soil pigment, the amount of red-brown plant coloring materials, the amount and nature of the vegetable indicators, and the proportion of colorless compounds present in the ammonia extract. The range of variation in these factors is beautifully illustrated by some data on page 243 of Alway and Blish's (2) paper. These authors were working with the very uniform soil type, the loess, the extremes differing mainly in rainfall. They find an average of 0.33 and 0.31 per cent of humus (gravimetric) respectively for the 3 to 6-foot section in the "Wauneta" and "Weeping Water" areas, while by colorimetric methods they obtain 0.52 per cent for the "Wauneta" area and 0.08 per cent for the "Weeping Water" area, or a difference of 500 per cent by the colorimetric method on soils having an almost identical humus content.

Briggs, Jensen and McLane (8) present evidence in their own paper which proves that certain of their humus determinations are unreliable. It is exceedingly improbable that the ratio of carbon to organic matter ever rises above 2. The European factor for the total organic matter of soils is carbon $\times 1.724$ (20, p. 15), which is decidedly lower than the 1.842 which I found for peats and 1.971 for unchanged vegetable materials (*cf.* Table XXXI). Using the European factor, we find that in soils Nos. 100, 123, 124 and 127, Briggs *et al.* obtain a greater percentage of humus than there was total organic matter in the soil. Hilgard (17, p. 132) states that this factor (1.724) will give results which are from 40 to 50 per cent higher than the humus content, or, in other words, that the ammonia-soluble materials represent from 50 to 60 per cent of the total organic matter. If this be the case, a very considerable proportion of the "humus" determinations of Briggs *et al.* are much too high. These authors fail to find a correlation in the case of the lemon groves between humus and mottle-leaf. In this instance the soil is all from the same locality and presumably from the same soil type, so that the (relative) humus percentages should be quite accurate, provided they were all matched against the same standard. The criticism of the use of the colorimetric method would, therefore, not apply with equal force in these experiments.

Inasmuch as these authors have apparently not recognized the weaknesses of the colorimetric method, the accuracy of their humus determinations is open to serious question. Whether the errors are great enough to vitiate entirely their correlations can be determined only by actual experiment, in which the humus is determined gravimetrically.

It does appear possible, however, that there is a positive correlation between soil pigment and mottle-leaf. It seems to me to be extremely improbable that this soil pigment plays any part in the nutrition of the citrus trees or the prevention of mottle-leaf, *per se*. The low nitrogen content, and the persistence of the soil pigment at considerable depth in the soil, seem to indicate that the soil pigment is not readily destroyed. May it not be possible, if the positive correlation between the color of the ammonia extract of soils and mottle-leaf of citrus trees holds true, that mottle-leaf is due to decreased bacterial activity in the soil, the amount of soil pigment being merely an indicator of bacterial activity?

Having shown that the humus contains approximately 50 per cent of carbon, a direct comparison of the "humus" extracted by NaOH and NH_4OH is possible. This is shown in Tables XXVII and XXVIII and in every instance sodium hydroxide extracted more humus than the ammonia.

It was thought possible after the carbon-humus ratio had been determined that a ratio might be found by which the "volatile matter" of peats and vegetable materials could be transposed into carbon. Such an attempt on a small scale is shown in Table XXXI. The factors used are the average factors for the determinations given. It will be noted again that the factor for the unchanged vegetable materials is practically 2, which would mean that the carbon content of the undissolved material is approximately the same as that of the "humus" dissolved by 4 per cent NH_4OH . It is to be hoped that someone will extend this series and see if the factors given are actually reliable over a large number of determinations.

V. *A comparison of the carbon and nitrogen dissolved from unleached soils by 2 per cent and by 4 per cent sodium hydroxide solutions.* It was the original intention to investigate the amounts of carbon dissolved by different concentrations of sodium and ammonium hydroxides. Interest, however, became diverted to other directions early in the progress of the work, but not until the data were practically complete for two concentrations of sodium hydroxide on the unleached soils. These are shown in Table XXIX. The 2 per cent NaOH solution has been used almost exclusively in the study of the organic matter of the soil carried out by the Bureau of Soils of the U. S. Department of Agriculture. The data available indicate that 2 per cent NaOH dissolves the same substances (*cf.* C/N ratios) in almost the same amount as does a stronger solution. However, the length of time during which the solution was in contact with the soil and the thoroughness of the stirring are factors which operate greatly to equalize the amount dissolved.

SUMMARY

Eight mineral soils, three peats, one muck and five samples of unchanged vegetable materials were selected and analyzed for total carbon and total nitrogen; for carbon and "humus" soluble in 4 per cent NH_4OH both before and after leaching the substances with 1 per cent HCl; for carbon and nitrogen soluble in 4 per cent NaOH both before and after leaching the material with 1 per cent HCl; for carbon and nitrogen extracted by 4 per cent NaOH from the residue remaining after the soil or vegetable materials which, without previous treatment, had been extracted with 4 per cent NaOH and subsequently leached with acid; for carbon and nitrogen extracted by the addition of water to the residue remaining from the preceding extraction and for nitrogen in the 1 per cent HCl ex-

tract. Colorimetric measurements were also made on the various solutions.

From a comparison of the data obtained in this series of analyses, the following conclusions are evident:

1. The "humus" extract of soils and peats is not a typical soil product, formed in the soil by the action of bacteria and fungi, for very similar extracts can be obtained from unchanged vegetable materials.
2. "Humus," the "matière noire" of Grandeau, does not consist entirely of a black compound, or compounds, but it also contains a large proportion of almost colorless substances, the presence of which is normally masked by the black color.
3. A 4 per cent solution of sodium hydroxide does not extract either the same substances or the same quantity of substances as does a 4 per cent solution of ammonium hydroxide.
4. From a given soil, after leaching with 1 per cent HCl, ammonium hydroxide will extract *less carbon* and at the same time *more color* than will a sodium hydroxide solution.
5. In general the forms of soil nitrogen appear to be quite similar in solubility to those forms occurring in unchanged vegetable materials, the only noteworthy difference being that vegetable materials contain a very considerable amount of nitrogen soluble in 1 per cent HCl while soils contain only a small quantity, and, on the other hand, the soils contain an appreciable amount of nitrogen quite insoluble in 4 per cent NaOH, in decided contrast to the unchanged vegetable materials.
6. All the mineral soils and the one calcareous peat tested, contained a *soil pigment* which is absent from the unchanged vegetable materials or the acid peats.
7. This soil pigment appears to be the only substance which can, with certainty, be said to be a true soil product. It is suggested that perhaps the soil pigment is of bacterial origin.
8. This soil pigment is intensely black, and contains only a relatively small proportion of the soil nitrogen. Therefore, it would appear to have but little importance, *per se*, in the problem of soil fertility.
9. It is extremely doubtful whether a specific "humification" of plant materials takes place in the soil.
10. Inasmuch as sodium hydroxide does not dissolve either the same substances or the same quantity of substances as does ammonium hydroxide, the common practice of comparing "humus nitrogen" determined in a 4 per cent NaOH extract, with "humus," obtained by 4 per cent NH_4OH , can give only meaningless results.
11. Inasmuch as the "humus" extract of soils is undoubtedly a mixture of organic compounds, many of which are colorless and in all probability are extracted from unchanged plant or animal materials, and inasmuch as the soil pigment present in this solution probably rarely exceeds

40 per cent of the "humus," a determination of the "humus" as ordinarily carried out, appears to be wholly without scientific justification. The European method of reporting "humus" by making a determination of total organic carbon, appears to be far preferable.

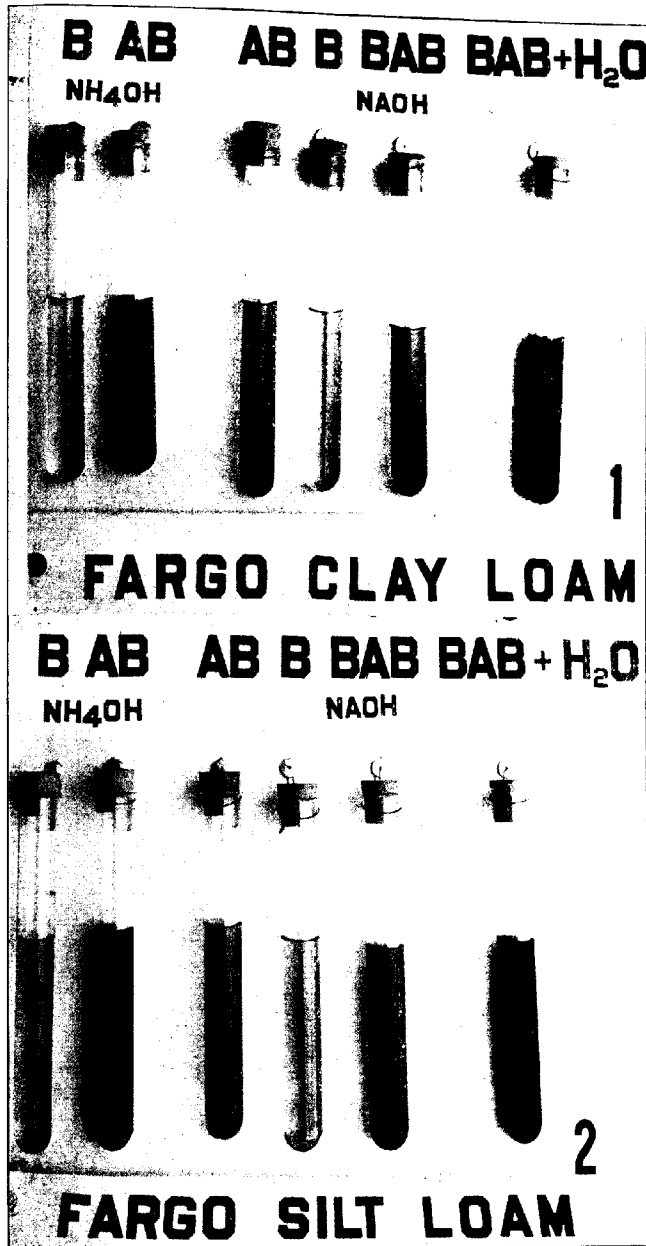
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PLATE I

- Fig. 1.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Fargo clay loam. Each tube contained 0.0042 gm. organic carbon per 50 c.c. volume.
- Fig. 2.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Fargo silt loam. Each tube contained 0.0046 gm. organic carbon per 50 c.c. volume.



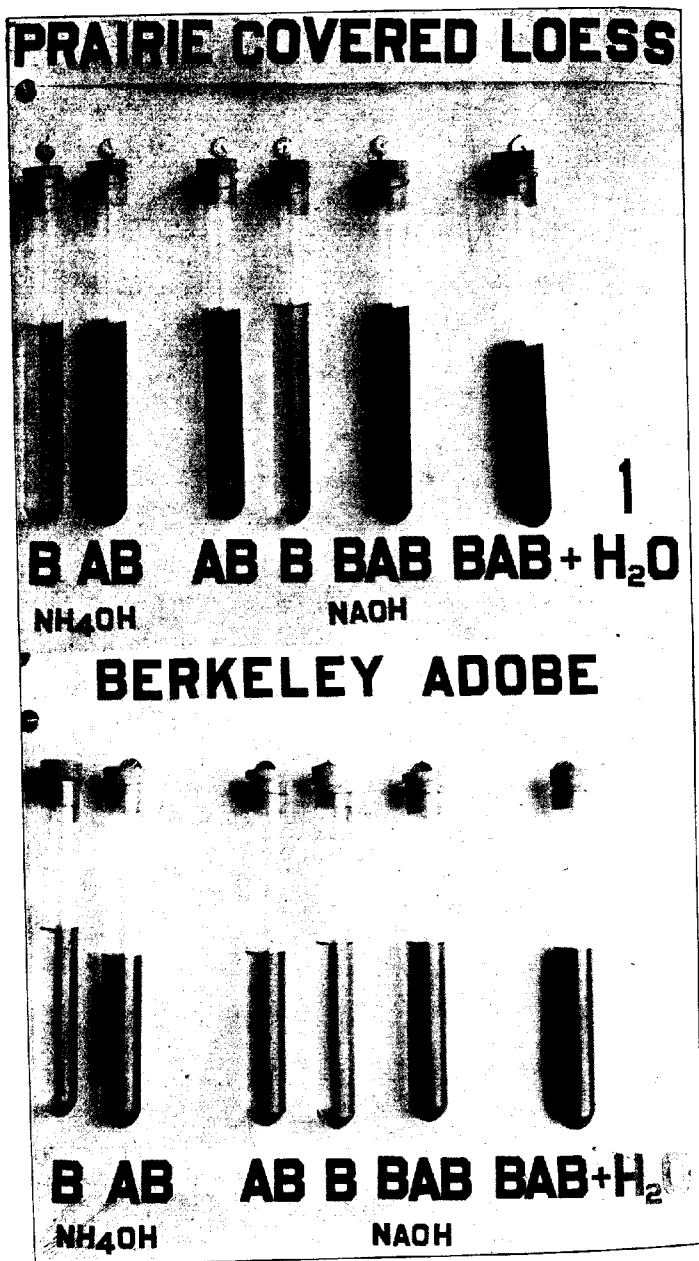


PLATE II

Fig. 1.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from prairie-covered loess. Each tube contained 0.0046 gm. organic carbon per 50 c.c. volume.

Fig. 2.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Berkeley adobe. Each tube contained 0.0030 gm. organic carbon per 50 c.c. volume.

THE INFLUENCE OF SALTS ON THE BACTERIAL ACTIVITIES OF THE SOIL¹

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Salts which may occur in soils and those applied to them in various operations influence the number, species, and activity of the microflora of a soil. These factors are in turn reflected by the yields obtained from the soil. Some substances applied to a soil serve as food for the growing plant; others increase plant growth but not through the direct furnishing of food. This latter effect may be due to a change brought about by the salt on the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified; this in turn may increase or decrease the crop produced upon the soil. Other substances may react chemically with constituents within the soil and in so doing liberate substances which can be directly utilized by the growing plant. Again, they may directly modify the microflora and fauna of the soil both as to numbers and physiological efficiency. Or, in some cases all three changes may be wrought by one and the same salt. The question, therefore, arises as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activity of the soil. Furthermore, there are millions of acres of land in arid America which contain varying amounts of soluble salts. Some of these soils contain such large quantities of these so-called "alkalies" that no vegetation is found upon them. Other soils contain only a medium amount of soluble salts and the vegetation is composed chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition can be carried on successfully only when we understand the influence of salts upon the growing plants and their action upon the biological, chemical, and physical properties of the soil.

¹ Contribution from the chemical and bacteriological departments of the Utah Agricultural Experiment Station.

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The purpose of this investigation is, therefore, to determine the relative toxicity of various substances found in or applied to a soil, as measured in terms of bacterial activities of the soil, also to determine the stimulating influence of various substances upon bacterial activity and the manner in which this stimulation is exerted. The results thus obtained can be directly compared with those obtained for higher plants. Then, if a correlation between the two (the lower and the higher plants) be obtained, it should give a quick method of testing not only alkali soils, but also other soils containing various soluble constituents. Furthermore, it makes possible the studying of balanced solutions within the soil by means of bacteriological tests, thus getting in a short time comparative results which with higher plants would be obtained only by an enormous amount of work and time. Knowledge thus obtained can be used in the reclaiming of the alkali lands of the arid West. A careful review of the literature has been made, and below is given the more recent important work dealing with the action of various salts upon the bacterial flora of the soil.

HISTORICAL

Much work has been done to determine the influence of calcium carbonate, especially when applied to acid soils, on the bacterial content and activity of the soil, but the conclusions reached have not always been concordant. Withers and Fraps (56) found that calcium carbonate added to a soil greatly accelerated nitrification and that it is especially desirable that it should be added where ammonium sulphate is being used as a fertilizer. Lipman's (32) work showed that calcium carbonate stimulated nitrification more than did gypsum, and that sodium chloride was injurious to nitrifying organisms, while ferrous sulphate in amounts from 10 to 100 mg. per 100 gm. of soil was without effect. Later, he and Brown (34) decided that both ammonification and nitrification were promoted by magnesia lime to a more marked extent than they were by non-magnesia lime. This, however was to a certain extent dependent upon the treatment and crop growing on the soil. Both ammonification and nitrification were accelerated by sodium nitrate. In a more recent work Lipman, Brown and Owen (36) found that small applications of calcium carbonate stimulated bacterial activity, but large applications had a detrimental effect upon ammonification.

In Owen's (44) experiments, magnesium carbonate was more efficient in promoting ammonification and nitrification than was either calcium or potassium carbonate. According to Engberding (10) ammonium sulphate, sodium nitrate, potassium nitrate and caustic lime all increase the bacterial content of the soil, but decrease its nitrogen-fixing powers. Krueger's (23) work indicated that calcium carbonate was more effective in promoting nitrification than was lime, the reverse was true with regard

to the putrefactive bacteria. The formation of ammonia from peptone was especially favored by calcium carbonate. Lyon and Bizzel (38) found that lime favored nitrification, as did also certain nodule-bearing legumes. Fischer (12) concluded that the presence of calcium carbonate in a nutritive solution favored the formation of protein nitrogen, while magnesium carbonate lessened the transformation of ammonia into protein nitrogen. Calcium oxide, however, exerted a much greater influence upon soil bacteria than did calcium carbonate.

Kellerman and Robinson's (20) results are of especial interest as they indicate that magnesium carbonate, applied in amounts exceeding 0.25 per cent to a soil fairly high in magnesium carbonate, positively inhibited nitrification, while calcium carbonate up to 2 per cent favored it, thus indicating that the lime-magnesia ratio is of great importance with regard to bacteria as well as the higher plants. These results have been confirmed by C. B. Lipman and Burgess (30a) in whose experiments magnesium carbonate was very toxic both in soil and solution to *Azotobacter chroococcum*, while calcium carbonate was never toxic even in quantities up to 2 per cent. Furthermore, calcium carbonate exerted a protective influence against the toxic properties of magnesium carbonate. The optimum ratio varied, depending upon the media.

Peck (46) studied the influence of a number of salts upon bacterial activity when applied to the soil, with the result that the carbonate, sulphate and phosphate of calcium were found to stimulate ammonification, while sodium nitrate depressed it; both potassium sulphate and calcium carbonate accelerated nitrification in soil. Brown (3) working with a typical Wisconsin drift soil found that the application of ground lime up to 3 tons per acre increased the number of bacteria in the soil, also the ammonifying, nitrifying and nitrogen-fixing powers of the soil. The increase was in every case nearly proportional to the limestone applied.

At times the increase noted in ammonification is due to the retention of the volatile ammonia by the carbonate, as is shown by Lemmermann's (24) results where the addition of calcium carbonate to a soil up to 1 per cent reduced the volatilization of ammonia, but calcium oxide had the opposite effect. Both calcium chloride and calcium sulphate reduced the loss of ammonia, but the chloride was the only salt of magnesium tested which had this effect. Potassium and sodium, chloride, sulphates and carbonates all reduced the absorptive powers of the soil. Paterson (45) studied the influence of a number of substances upon nitrification with the result that caustic lime was found practically to stop all nitrification. Calcium carbonate promoted it, as did also magnesium carbonate; gypsum was less effective, while ferric hydrate had a very favorable effect. Sodium chloride, on the other hand, had a distinctly injurious effect.

Kelly (21) studied the effect of calcium and magnesium carbonate alone and in combination upon ammonification and nitrification. In his work calcium carbonate only slightly stimulated ammonification of dried blood, but it had a marked stimulating effect upon nitrification. The magnesium carbonate was found to be toxic to both groups of organisms. No antagonism was found to exist between calcium and magnesium. Later, when working with Hawaiian soils, he (22) reports a stimulation for both. The results, however, varied with different soils and he considers the lime-magnesia ratio of little importance as regards the ammonifying and nitrifying organisms. Allen's (1) conclusion is that large quantities of limestone must be applied to a non-calcareous soil in order to bring its nitrifying powers up to those of natural calcareous soils.

Peterson (47) and Wollny (57) found that lime increased the carbon dioxide given off from soils, and Ebermeyer (8), Hilgard (19), and Hartwell and Kellogg (18) proved conclusively that lime increases the decay taking place in a soil.

Chester (4, 5) showed that lime increased the number of bacteria in soil, the increase being proportional to the lime applied up to 4,000 pounds per acre. He considered the effect as being due to the lime giving to the soil a more favorable reaction for the growth of bacteria and not to its direct action upon the organisms themselves.

These findings were later confirmed by Fabricius and von Feilitzen (11), Engberding (10), Ehrenberg (9) and Fischer (12).

Lime not only increases the number of organisms in a soil, but it increases the ammonifying powers of the soil as is seen from the work of Remy (49), Ehrenberg (9) and Voorhees and Lipman (54).

The literature dealing with the influence of lime upon the nitrifying organisms is voluminous. No attempt is made here to refer to all of it, for in most cases the experiments were conducted on soils which were acid and the lime supplied neutralized the acidity of the soil, thus give the necessary neutral reaction for the action of the nitrifying organisms. Such results give little, if any, idea of the direct stimulating or toxic influence of calcium. Furthermore, the work has recently been summarized by Brown (3) who concludes that the application of lime increased nitrate production from ammonium sulphate and dried blood, the gain being almost proportional to the quantity of lime applied. This, in turn, was found to bear a close relationship to the number of organisms developing on synthetic agar.

Gypsum is a strong soil stimulant and in most cases it greatly increases the crop yield. This beneficial effect may be due to its liberation of potash or to the supplying of sulphur for the direct nutrition of the plant. At other times it may react with the ammonia formed by the ammonifying organisms, the neutral ammonium sulphate so formed being

readily nitrified. There is also the possibility that calcium sulphate acts as a direct stimulant to the microorganisms of the soil. The literature is meager and inconclusive on this phase of the subject.

Severin (52) concludes from his work that gypsum not only prevents the loss of ammonia from manure, but it increases the speed of decomposition from 10 to 20 per cent, while Paterson (45) states that gypsum slightly increases nitrification in soil as determined by laboratory experiments. Prior to this, Pichard (48) had shown that the sulphates of calcium, potassium, and sodium promote nitrification.

Opposite results are reported by Dezani (6) who found that gypsum in amounts varying from 0.5 to 2 per cent had no appreciable effect on nitrification, while the results obtained by Lipman et al (33) varied and were inconclusive.

According to Lipman (25) calcium chloride in solutions accelerated the action of ammonifiers, and it is interesting to note that in a later work he (26) failed to find antagonism between either calcium and magnesium or calcium and sodium. The chlorides of calcium, magnesium, potassium, and sodium were found to be toxic in the order named. Marked antagonism exists between calcium and potassium, magnesium and sodium, and potassium and sodium. Sea water was found to be a physiologically balanced solution for *Bacillus subtilis*.

Many writers have made great claims for iron sulphate as a fertilizer. A goodly number of these have been made by individuals who would profit by its sale, but even when we ignore these cases there are still many cases in which it has produced good results. The composition of the crop usually indicates that iron sulphate influences the phosphorus (14) metabolism of the plant, and it is hard to see how this is possible unless it be that the iron stimulates the bacterial activity, which in turn liberates phosphorus from its insoluble forms within the soil. It has already been noted that Lipman (32) found ferrous sulphate to have but little effect upon nitrification, but his results were not conclusive. Gutfroy (16) found sulphate of iron decidedly beneficial to oats, less beneficial in the case of rye, without action on rye grass and harmful to wheat. He concluded that its action must be due to its influence on the biological processes of the soil. According to Paterson and Scott (45) ferric hydrate has a distinctly beneficial effect upon nitrification. In this latter case its action could be due to its serving as a base. According to Lipman and Burgess (30) the ammonifiers are more sensitive to iron sulphate than are the nitrifiers, for while the latter were stimulated by small amounts of iron sulphate it was toxic to the former in all concentrations tested.

Magnesium compounds usually stimulate bacterial activities to a greater extent than do calcium compounds, as has been noted in some of the literature already cited, but Engberding's (10) results showed that,

while magnesium sulphate stimulated bacterial activities, it was not as effective in this regard as was ammonium sulphate, sodium nitrate, or potassium sulphate. The work of Makrinov (39) is of interest as he found pure magnesium carbonate a very suitable substance on which to grow the nitrous organism. Furthermore, magnesium carbonate had a very beneficial effect on the physiological action of the organism. Kellerman and Robinson (20), on the other hand, found that magnesium carbonate when applied to a soil already rich in magnesium carbonate positively inhibited nitrification if the quantity added exceeded 0.25 per cent. We have here an apparent contradiction, but it may be due to the different conditions of the experiments, as one investigator was working with cultures of the organisms while the other was using the soil with its complex flora. Furthermore, it is quite possible that magnesium carbonate may be without effect upon or even accelerate the growth and activity of the nitrosomonas and yet inhibit the nitrobacter.

Manganese stimulates higher plants and in many cases acts as a positive catalyzer. Skinner and Sullivan (53) conclude from some of their experiments that manganese salts increase the oxidation in some soils. But while Montanan (42) found that manganese carbonate, sulphate, and dioxide greatly stimulated nitrification he attributed it to either the direct or the indirect furnishing of oxygen to the nitrifying organisms, and not to any catalytic effect of the manganese itself. Beijerinck (2) observes that some soil organisms have the power of oxidizing manganous carbonate. Olaru (43) found that manganese in the proportion of one part to 200,000 parts of nutritive media greatly increases nitrogen fixation and he considers it quite likely that the increased yield obtained after the application of manganese compounds to a soil is due to its accelerating the action of the nitrogen-fixing organisms of the soil.

Potassium is essential for the nutrition of both the higher and lower forms of plant life. Hence, it is to be expected that when added to a medium poor in potassium it will increase bacterial growth, but like many other true nutrients may become toxic if present in too great a concentration. As already noted, Engberding (10) states that potassium sulphate caused a slight increase in the bacterial content of a soil. While Peck (46) found it to increase nitrification in soils, Renault (50) claims that slow ammonification and subsequent nitrification is always accompanied by a low percentage of potash. Dumont's (7) experiments showed that potassium carbonate, added to a soil at the rate of from 1 to 2.5 gm. per 1,000 gm. of soil, markedly increased nitrification, but that larger applications of the salt progressively diminished the rate of nitrification, while the addition of 8 gm. to 1,000 gm. of soil completely checked it. Lumia (37) concluded that potassium chloride and sulphate were nearly as effective in promoting the activity of alcoholic ferments as were phosphates.

Fred and Hart (13) found that both calcium and potassium sulphates increased ammonification in solution and that the sulphates of potassium, calcium and magnesium each increased the evolution of carbon dioxide from soil. But from the results obtained with different salts, they conclude that the addition of the potassium ion did not materially increase ammonification in the soil examined.

Sodium salts are often used as fertilizers and with good results. Furthermore, many alkali soils contain sodium salts in quantities sufficient to be toxic to both the higher and the lower plants. For these reasons many investigations have been conducted to determine the influence of sodium compounds upon higher plants, and a considerable number have had as their object the determination of their influence upon soil bacteria.

As early as 1834 Warington (55) showed that the presence of 0.032 per cent of sodium bicarbonate distinctly retarded nitrification, and that in the presence of 0.096 per cent nitrification was very slight. Schlösing (51) had added various salts to the soil in quantities not exceeding 485 parts per million with no apparent effect upon nitrification. However, Deherain found that common salt commenced to be harmful when it exceeded one-thousandth of the weight of soil, and when larger quantities are applied nitrification almost ceased. According to the same observer sodium nitrate may stop nitrification for a time, but later it recommences. Lipman (32) showed that there was a distinct diminution of nitrates produced as the application of sodium chloride was increased, and when 0.1 per cent was added nitrification was greatly diminished. Later he (35) and others found that sodium nitrate increased the accumulation of nitrates in a soil. They found, however, a certain periodicity in the accumulation of nitrates which would account for the different results reported by various investigators, and in later investigations they (33) concluded that at times sodium nitrate stimulates ammonification. McBeth and Wright (40) found that carbonates, chlorides and sulphates inhibited nitrification and that the former were more injurious than the latter.

The most far-reaching and systematic work which has been reported on the influence of salts upon bacterial activity is the excellent work by C. B. Lipman (27) who demonstrated that ammonification is inhibited by sodium chloride, sodium sulphate and sodium carbonate. The points at which the salts became toxic are: for sodium chloride, between 0.1 per cent and 0.2 per cent; for sodium sulphate, 0.4 per cent; and for sodium carbonate, 2.0 per cent. A stimulating influence was noted in the case of sodium carbonate, but not in the case of the sulphate or of the chloride. The points at which they became toxic to nitrifiers (28) were found to be: for sodium carbonate, 0.025 per cent; for sodium sulphate, 0.35 per cent; and for sodium chloride, less than 0.1 per cent.

All except the carbonate acted as a stimulant in lower concentrations. Later Lipman and Sharp (31) found the point at which sodium chloride became toxic to nitrogen-fixing organisms in soil to be from 0.5 to 0.6 per cent; sodium sulphate, at 1.25 per cent; and sodium carbonate, at 0.4 to 0.5 per cent. Sodium chloride was the only one which acted as a stimulant. Recently Lipman (29) has demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium sulphate; between sodium chloride and sodium carbonate; and between sodium sulphate and sodium carbonate.

At times the stimulating effect, or even the toxic influence, may be due to the liberation of substances within the molecule of the salt, as for example, in the case of the sulphates it may be the sulphur, but no attempt has been made in this summary to cover the literature on this important phase of the subject.

EXPERIMENTAL

The soil used in this work was that of the College Farm which is of a sedimentary nature. It was deposited by streams flowing into the valley laden with debris derived from the nearby mountains, which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil is given in Table I.

TABLE I
PHYSICAL AND CHEMICAL COMPOSITION OF SOIL.

Physical	Per cent	Chemical	Per cent
Coarse Sand (above 1 mm.).....	17.69	Insoluble Matter	66.69
Fine Sand (1 to 0.03 mm.).....	37.39	Potash (K_2O)	0.55
Coarse Silt (0.03 to 0.01 mm.).....	15.19	Soda (Na_2O)	0.49
Medium Silt (0.01 to 0.003 mm.).....	10.36	Lime (CaO)	7.41
Fine Silt (.003 to 0.001 mm.).....	10.32	Magnesia (MgO)	4.15
Clay (below .001 mm.).....		Ferric Oxide (Fe_2O_3)	2.93
Moisture and Loss	9.05	Alumina (Al_2O_3)	3.49
		Phosphoric Pentoxid (P_2O_5)	0.25
		Sulphur Trioxid (SO_2)	0.07
		Carbon Dioxid (CO_2)	7.62
		Humus	2.18
		Total Nitrogen	0.15

The soil used, therefore, was a sandy loam very high in acid-soluble constituents, but the water-soluble constituents were not excessive. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium and there was a fairly large quantity of iron present. In fact all of the elements of plant-food were present in abundance, with the exception of nitrogen which was low. The soil was very productive and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The nitrogen-fixing powers of the soil were above the average and previous work had shown it to have an intensely interesting bacterial flora.

Several hundred pounds of the soil were thoroughly mixed, stored in a large box, and kept as near field conditions as possible so that all the work could be done on the same soil. As the soil was needed in the work, portions were brought to the laboratory, air-dried in the dark, then weighed in 100-gm. portions into sterile covered tumblers. To each of these was added 2 gm. of dried blood. The whole was then carefully mixed and the salt in most cases added from a carefully standardized stock solution. This, together with sufficient sterile distilled water to make the moisture content up to 18 per cent, was thoroughly mixed in the soil. Each series, together with sterile blanks, was incubated at 28° to 30° C. for 4 days and then the ammonia determined by adding magnesium oxide and distilling into standard acid. In every case at least four determinations were made with each concentration of the salt, and, in the absence of agreement between determinations, the series was repeated so that the results as herein reported are in every case the average of four or more closely agreeing determinations. Hence, experimental error has been reduced to as near a minimum as possible in this kind of work.

The solutions of the salts were prepared by weighing gram-molecular quantities of Merck's best grade of the respective salts into 1,000 c.c. of sterile distilled water and then quantitatively determining the amount present. In those cases in which the analysis showed the concentration wrong, it was corrected, so that we have a definite knowledge of the quantity of salt added to the soil as the varying results reported by different investigators can in many cases be interpreted by the unknown variation in salts added. The solution thus prepared was then added to the soil in such quantities as to make the amount of the anion and of the cation the same and directly comparable the one with the other. The comparatively insoluble salts—calcium carbonate, calcium sulphate, etc.—were carefully weighed and intimately mixed with the soil. The arranging of the work in this order gives us as nearly absolute results as can be obtained by the present bacteriological methods, and at the same time gives us directly comparable results, which after all is what we have to look for in this work.

The salts tested were the chlorides, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron.

Ammonification

Influence of Sodium Salts: The compounds used in this series were sodium chloride, sodium sulphate, sodium nitrate and sodium carbonate. They were used in concentrations such that equivalent quantities of sodium in the various forms could be directly compared. The strengths varied from 0 to 5060.4 parts per million of soil, and represented the actual proportion of sodium in the various forms applied to the soil.

The results are reported in the form of per cent, considering the ammonia produced in the untreated soil in each case as 100. This method of reporting the results makes them more directly comparable than if stated as milligrams of ammonia formed in 100 gm. of soil. It did not, however, materially change the numerical values, as the average ammonifying power of the untreated soil was 98 mg. of ammonia per 100 gm. of soil. The results are given in Table II and in every case are the average of at least four and sometimes of several times this number of closely agreeing determinations; so they should represent very closely the comparative influence of the various sodium salts upon ammonification.

TABLE II
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING
2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF
SODIUM SALTS, THE UNTREATED SOIL TAKEN AS 100 PER CENT

Sodium—Parts per Million	Per cent of Ammonia Formed in the Presence of			
	NaCl	Na ₂ SO ₄	NaNO ₃	Na ₂ CO ₃
None	100.0	100.0	100.0	100.0
3.6	106.0	96.0	104.6	105.6
7.2	106.1	95.2	103.7	104.4
14.4	97.9	100.0	102.3	106.8
28.8	92.4	97.6	104.4	107.4
57.5	88.5	96.8	107.8	107.6
115.0	73.4	96.0	94.2	108.9
230.0	67.3	92.2	90.6	110.1
460.0	57.6	86.5	81.7	108.3
920.1	40.6	72.4	75.9	103.8
1380.1	29.9	66.8	56.0	102.9
1840.1	24.1	62.8	54.0	101.2
2300.1	18.0	55.2	46.6	101.4
2760.2	18.0	51.6	46.0	94.7
3220.2	16.2	46.0	41.5	92.5
3680.3	16.1	42.8	41.5	88.6
4140.3	11.8	40.5	40.1	80.5
4600.3	11.5	33.2	37.9	67.1
5060.4	11.2	32.0	35.4	65.6

It may be seen that sodium chloride when added to the soil in the proportion of 7 parts of sodium per million of soil acts as a stimulant to the ammonifying organism of that soil. Sodium sulphate, on the other hand, even in the lowest concentration tested—3.6 parts per million of sodium—failed to stimulate the ammonifying organisms. Sodium nitrate stimulates in low concentration and has its greatest effect when 57.5 parts per million of sodium in the form of sodium nitrate is added. The carbonate has its greatest stimulating influence when 230 parts per million of sodium in the form of sodium carbonate is added to the soil. This compound continues to stimulate until the concentration exceeds 2,300 parts per million. The results, as a whole, indicate that it is the

electro-negative ion which acts as the stimulant and not the sodium ion. The sodium chloride becomes toxic when only 14.4 parts per million of sodium as sodium chloride is added to the soil, while the sodium sulphate does not become toxic until twice this quantity is added. The nitrate does not become toxic until eight times as much has been added as is required of the chloride to retard ammonification, while it requires 190 times as much of the carbonate as of the chloride to retard ammonification.

These results confirm those obtained by C. B. Lipman (27) for the relative toxicity of the chloride, sulphate, and carbonate are in the order found by him. Furthermore, it shows that this order is held by these salts where applied to soils differing widely in composition. The results throughout are similar to Lipman's with the exception of the stimulation by sodium chloride in this series, and it is quite likely that

TABLE III
PERCENTAGE OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING
2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS
OF SODIUM SALTS IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Weight in 100 gm. of Soil	Per cent of Ammonia Formed in the Presence of			
	NaCl	Na ₂ SO ₄	NaNO ₃	Na ₂ CO ₃
None	100.0	100.0	100.0	100.0
156×10 ⁻⁷	106.0	95.2	104.6	104.4
312×10 ⁻⁷	106.1	100.0	103.7	106.8
625×10 ⁻⁷	97.9	97.6	102.3	107.4
125×10 ⁻⁶	92.4	96.8	104.4	107.6
25×10 ⁻⁵	88.5	96.0	107.8	108.9
5×10 ⁻⁴	73.4	92.2	94.2	110.1
1×10 ⁻³	67.3	86.5	90.6	108.3
2×10 ⁻³	57.6	72.4	81.7	103.8
4×10 ⁻³	40.6	62.8	75.9	101.2
6×10 ⁻³	29.9	51.6	56.0	94.7
8×10 ⁻³	24.1	42.8	54.0	88.6
10×10 ⁻³	18.0	33.2	46.6	67.1

he would have observed this, even though working with soils widely different from those used in this work, had he used lower concentrations of the salt. We find 0.2 per cent the lowest concentration used by him, and this reduced the ammonifying powers to 30 per cent normal which is only slightly lower than the results obtained in this work when that concentration of sodium chloride was used. Furthermore, the results are in keeping for the nitrate obtained by Lipman and others.

The toxicity of the chloride increases much more rapidly than does the toxicity of the other compounds. The highest concentration tested, 5000 parts per million, nearly stopped ammonification where the chloride was added, but the sulphate had reduced it to only 32 per cent, the nitrate to 35.4 per cent, and the carbonate to only 65.6 per cent. The

results point to the conclusion that it is the anion and not the cation which exerts the main toxic influence on the ammonifiers. The action of sodium sulphate and sodium nitrate after the first stimulating influence are nearly paralleled throughout all concentrations tested.

The work was so planned that equal molecular portions of the various salts could be compared, as shown in Table III.

A given molecular quantity of sodium chloride is much more toxic to ammonifying organisms than is an equivalent molecular proportion of sodium sulphate, and this in turn is more toxic than is an equivalent molecular proportion of sodium nitrate. Sodium carbonate is less toxic than any of the other salts tested. When 2×10^{-3} mol. of sodium chloride

TABLE IV
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL TO WHICH WERE ADDED 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND DIFFERENT FORMS OF POTASSIUM SALTS

Potassium—Parts per Million	Per cent of Ammonia Formed in the Presence of			
	KCl	K ₂ (SO ₄)	KNO ₃	K ₂ CO ₃
None	100.0	100.0	100.0	100.0
6.1	94.6	99.6	102.2	103.8
12.2	95.8	98.4	100.4	105.8
24.4	91.9	98.4	101.8	107.7
48.9	89.1	98.9	99.1	108.0
97.8	85.9	90.9	98.3	106.1
195.5	80.5	87.0	95.0	106.1
391.0	76.3	86.0	89.5	106.1
782.0	64.2	74.1	83.5	107.2
1564.0	50.5	56.6	68.7	108.0
2346.0	42.7	53.7	50.7	105.8
3128.0	38.6	53.7	51.1	100.0
3910.0	27.9	51.8	55.4	98.1
4692.0	22.3	53.7	49.0	88.5
5474.0	22.3	51.5	41.6	89.2
6256.0	18.4	50.8	34.4	78.5
7038.0	15.6	41.8	30.5	75.8
7820.0	14.8	37.5	26.5	68.3
8602.0	14.8	30.7	25.8	54.6

is added to a soil, it reduces the soil's ammonifying power to 57.6 per cent. Equivalent quantities of sodium sulphate reduce it to 72.4 per cent, and of sodium nitrate to 81.7 per cent, while sodium carbonate in this proportion acts as a stimulant. In the highest concentrations of the salts tested, 10×10^{-3} mol., there was produced in the presence of sodium chloride 18.0 per cent of ammonia, while approximately twice as much ammonia was produced in the presence of the sulphate, three times as much in the presence of the nitrate, and four times as much in the presence of the carbonate.

Sodium chloride which has the lowest molecular weight is the most toxic of the sodium salts used, but the next in order of molecular weight is not the next in order of toxicity. However, the toxicity of the sodium salts varies with the electro-negative ion with which sodium is combined,

and is due to a physiological influence exerted by it upon the protoplasm of the organisms in addition to the osmotic effect.

Influence of Potassium Salts

The compounds used in the potassium series were the chloride, sulphate, nitrate and carbonate. The concentrations were the same as those used in the sodium series. The results as given in Table IV are the average of four or more closely agreeing determinations.

Neither potassium chloride nor potassium sulphate stimulates the ammonifiers in any of the concentrations tested. In this respect the potassium salts differ from the sodium salts, for the sodium chloride in the low concentrations acts as a stimulant. Potassium nitrate in the lowest concentrations stimulates, yet it is not as effective as is the corresponding sodium salt. Potassium carbonate exhibits its highest point of stimulation when 1500 parts per million of potassium in the form of this salt has been added to the soil.

TABLE V

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF POTASSIUM SALTS IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Weight in 100 gm. of Soil	Per cent of Ammonia Formed in the Presence of			
	KCl	K ₂ SO ₄	KNO ₃	K ₂ CO ₃
None	100.0	100.0	100.0	100.0
156x10 ⁻⁷	94.6	98.4	102.2	105.8
312x10 ⁻⁷	95.8	98.4	100.4	107.7
625x10 ⁻⁷	91.9	98.9	101.8	108.9
125x10 ⁻⁶	89.1	90.9	99.1	106.1
25x10 ⁻⁵	85.9	87.0	98.3	106.1
5x10 ⁻⁴	80.5	86.0	95.0	106.1
1x10 ⁻³	76.3	74.1	89.5	107.2
2x10 ⁻³	64.2	56.6	83.5	108.0
4x10 ⁻³	50.5	53.7	68.7	100.0
6x10 ⁻³	42.7	53.7	50.7	88.5
8x10 ⁻³	38.6	50.8	51.1	78.5
10x10 ⁻³	27.9	37.5	55.4	68.3

Both potassium chloride and potassium sulphate are toxic in the lowest concentrations tested, and it is doubtful if even in lower concentrations they would act as stimulants. Potassium in the form of the nitrate does not become toxic until 48.9 parts per million have been added to the soil. The carbonate does not become toxic until nearly 4000 parts per million have been added to the soil. The toxicity of potassium in the form of the chloride and the sulphate rapidly increases with increasing concentrations until, when 1,564 parts per million have been added to the soil, the ammonifying power has been reduced to half its original value. The nitrate at this concentration has reduced it to 68.7 per cent, while the carbonate at this same concentration exerts its highest stimu-

lating effect, or produces 108 per cent of ammonia. Above this concentration the toxicity of all the compounds increases in about the same order, so that when 8,602 parts per million of potassium in the form of the chloride has been added to the soil, it produces only 14.8 per cent as much ammonia as it does in the untreated soil. Twice as much is found in the soils containing the sulphate and nitrate, and three times as much in the soil receiving the carbonate. The results as a whole indicate that a greater effect is produced by the anion than by the cation.

The influence of equivalent molecular proportions of the various potassium salts is given in Table V.

A given molecular quantity of potassium chloride is much more toxic to the ammonifiers than is an equivalent molecular quantity of the sulphate, nitrate, or carbonate, the toxicity of the compounds varying in the order named. If 4×10^{-3} mol. of potassium chloride or sulphate be added to 100 gm. of soil, the ammonifying power is reduced to about half normal. This quantity of carbonate is without effect. In the highest concentration of the salts used, 10×10^{-3} , the chloride reduces the ammonifying powers to 27.9 per cent, the sulphate to 37.5 per cent, the nitrate to 55.4 per cent, and the carbonate to 68.3 per cent.

The toxicity of the potassium salts is governed by the electro-negative ion combined with the potassium, and where this is an ion of a strong acid the toxicity is much greater than where it is the ion of a weak acid, thus indicating that the osmotic pressure of the solution, while not the only factor, plays no small part in the observed action.

Influence of Calcium Salts

The compounds used in this series were calcium chloride, calcium nitrate, calcium sulphate and calcium carbonate. The first two were added to the soil according to the usual method, from a standard solution, while the sulphate and carbonate were weighed into the soil, carefully mixed, and then treated in the regular manner.

A number of determinations were made in each case and compared with sterile blanks, so that the results as reported in Table VI are the average of four or more closely agreeing determinations.

Neither the chloride nor the nitrate of calcium stimulated in any of the concentrations tested. The sulphate stimulates in all concentrations below 200 parts per million, while the carbonate, with the exception of the first two concentrations, stimulated in every case. This tends to indicate that even a calcareous soil, such as is being used in this work, may be stimulated in so far as its ammonifying powers are concerned by the addition of limestone. Furthermore, the stimulation is quite marked, for we find the addition of 2×10^{-3} mol. of calcium carbonate to 100 gm. of this soil raises its ammonifying powers to 112.2 per cent.

Both the chloride and the nitrate are toxic in the lowest concentrations tested, while the sulphate does not become toxic until the concentration exceeds 400 parts per million. This would seem to indicate that its influence upon the physical properties of the soil must have something to do with its effect, for the soil solution would be saturated with the sulphate before this concentration was reached. After this first toxic effect, however, the ammonifying powers remained the same even where the largest quantities of calcium sulphate had been applied to the soil.

TABLE VI
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL TO WHICH WAS
ADDED 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS
AND FORMS OF CALCIUM SALTS

Fraction of Molecular Wgt. in 100 gm. Soil	Calcium—Parts per Million	Per cent of Ammonia Formed in Presence of			
		CaCl ₂	CaSO ₄	Ca(NO ₃) ₂	CaCO ₃
None	None	100.0	100.0	100.0	100.0
78x10 ⁻⁷	3.13	95.7	100.3	91.2	97.3
156x10 ⁻⁷	6.26	94.9	100.6	91.0	97.6
312x10 ⁻⁷	12.52	87.6	102.6	90.0	101.2
625x10 ⁻⁷	25.04	86.7	103.2	87.1	109.4
125x10 ⁻⁶	50.08	83.0	103.5	86.2	108.4
25x10 ⁻⁵	100.16	70.6	102.0	81.0	107.3
5x10 ⁻⁴	200.32	59.7	103.2	74.6	108.7
1x10 ⁻³	400.64	59.4	98.8	66.0	110.7
2x10 ⁻⁴	801.28	41.8	98.0	76.2	112.2
3x10 ⁻⁴	1201.92	38.5	98.6	75.8	111.5
4x10 ⁻⁴	1602.56	34.2	98.8	68.0	109.7
5x10 ⁻⁴	2003.20	32.1	91.0	60.4	110.4
6x10 ⁻⁴	2403.84	32.1	93.6	62.7	108.7
7x10 ⁻⁴	2804.48	25.4	97.4	61.8	108.7
8x10 ⁻⁴	3205.12	24.2	95.7	51.8	114.6
9x10 ⁻⁴	3605.76	23.3	96.6	37.8	104.8
10x10 ⁻⁴	4006.40	19.7	95.1	27.9	104.8
11x10 ⁻⁴	4407.04	19.1	96.5	22.3	107.6

In the presence of 4,407 parts per million of calcium in the form of the chloride the ammonia produced in the soil had been reduced to 19 per cent of the normal, while an equivalent quantity of the nitrate reduced it to 22 per cent. The sulphate at this concentration had reduced it only 3.5 per cent, while the carbonate was still stimulating. The influence of the calcium salt is therefore almost entirely governed by the electro-negative ion with which the calcium is combined.

It can be seen from these results that the addition of even 1 per cent of calcium carbonate to this soil, which already contains over 12 per cent of calcium carbonate, has a beneficial effect upon the ammonifying flora of the soil.

Furthermore, a given molecular quantity of calcium chloride is more toxic to the ammonifying organisms than is an equivalent molecular quantity of the nitrate, and this in turn is more toxic than the sulphate,

and the sulphate more so than the carbonate. It may be noted that 2×10^{-3} mol. of calcium chloride reduces the ammonifying powers of the soil to less than one-half their original, while an equivalent quantity of the sulphate is only slightly toxic. The carbonate in this concentration acts as a stimulant. The calcium compounds when applied to a soil in molecular quantities are in the order of decreasing toxicity calcium chloride, calcium nitrate, calcium sulphate, and calcium carbonate.

Influence of Magnesium Salts

The compounds used in the magnesium series were the chloride, sulphate, nitrate and carbonate of magnesium. The last named was applied to the soil in the form of a dry powder, while all of the others were added in the usual manner. The results representing the average of four or more closely agreeing determinations are given in Table VII.

TABLE VII
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING AMOUNTS AND KINDS OF MAGNESIUM SALTS, THE UNTREATED SOIL BEING CONSIDERED AS PRODUCING 100 PER CENT

Fraction of Molecular Wgt. in 100 gm. Soil	Magnesium— Parts per Million in the Soil	Per cent of Ammonia Formed in Presence of			
		MgCl ₂	MgSO ₄	Mg(NO ₃) ₂	MgCO ₃
None	None	100.0	100.0	100.0	100.0
78×10^{-7}	1.9	100.7	99.9	95.6	100.5
156×10^{-7}	3.8	99.0	98.7	90.7	101.6
312×10^{-7}	7.6	98.0	101.3	88.3	103.4
625×10^{-7}	15.2	96.6	104.5	82.3	103.5
125×10^{-6}	30.4	95.7	97.9	80.0	101.0
25×10^{-5}	60.8	95.6	94.5	92.0	101.0
5×10^{-4}	121.6	86.9	92.3	81.7	95.6
1×10^{-3}	243.2	74.9	84.1	83.0	91.3
2×10^{-3}	486.4	64.2	82.8	83.6	94.5
3×10^{-3}	729.6	64.2	82.8	75.1	89.8
4×10^{-3}	972.8	57.6	77.9	72.3	86.7
5×10^{-3}	1216.0	41.7	77.5	69.5	79.8
6×10^{-3}	1459.2	41.5	77.2	69.1	80.7
7×10^{-3}	1702.4	37.6	77.0	57.9	75.4
8×10^{-3}	1945.6	36.3	51.3	50.7	77.4
9×10^{-3}	2188.8	28.9	48.4	39.5	74.4
10×10^{-3}	2432.0	19.3	43.6	33.8	71.9
11×10^{-3}	2675.2	15.2	36.2	27.2	67.1

Both the sulphate and the carbonate of magnesium increase the ammonifying powers of the soil, both reaching their highest point of stimulation at about the same concentration. There is, however, this significant difference—the sulphate becomes toxic when slightly higher quantities are used, while the carbonate does not become toxic until 60.8 parts per million of magnesium have been added to the soil.

As in the calcium series neither the chloride nor the nitrate stimulate in any of the concentrations tested and the nitrate is quite toxic even in the lowest concentrations used. The toxicity of the chloride is just per-

ceptible in the second concentration tested. The toxicity of the chloride does, however, increase more rapidly than does that of the nitrates, for when 5×10^{-3} mol. of magnesium chloride is added to 100 gm. of the soil, its ammonifying powers are reduced to 41.7 per cent, while an equivalent quantity of the nitrate reduces it to 69.5 per cent. At this same concentration the sulphate and carbonate reduce it to 77.5 per cent and 79.8 per cent, respectively. The largest quantity of the magnesium salts added, 11×10^{-3} mol. in 100 gm. of the soil, reduces the ammonia found in the case of the chloride to 15.2 per cent, the nitrate to 27.2 per cent, the sulphate to 36.2 per cent, and the carbonate to 67.1 per cent.

The depressing effect of the magnesium sulphate and carbonate upon the ammonifying flora is gradual and uniform, while that of the chloride, and especially that of the nitrate, are very irregular.

It would appear from these results that the addition of 0.4 per cent of magnesium to this soil has a deleterious effect upon the ammonifying organisms of the soil. This must not, however, be taken as the exact quantity which is toxic to ammonifying organisms, for it must be borne in mind that this soil normally carries over 8 per cent of magnesium carbonate.

Influence of Manganese Salts

The quantity of manganese present varies greatly in different soils and in some cases it is added as a soil amendment. Furthermore, many experiments have shown it to increase plant growth when applied to a soil, but very little has been done to determine its influence upon the bacterial activity of the soil. Therefore determinations were made of the ammonia produced in a soil to which varying quantities of manganous chloride, manganous sulphate, manganous nitrate and manganous carbonate had been added. The results so obtained as the average of a great number of closely agreeing determinations are given in Table VIII.

With the possible exception of the chloride, all of the manganese salts tested were strong stimulants to the ammonifying organisms of the soil. The extent of the stimulation and the quantity of the salt required to produce the maximum stimulation varies greatly with the salt used. Probably the chloride of manganese stimulates slightly in the very lowest concentration tested, while 17.2 parts per million of manganese in the form of the sulphate must be applied to produce the maximum stimulation. However, when this quantity is applied to the soil there accumulates in that soil one-fourth more ammonia than in the untreated soil. The concentration at which the nitrate reaches its maximum stimulation is the same as that for the sulphate, but the nitrate is not nearly as strong a stimulant as is the sulphate. The carbonate, with two exceptions, stimulates in all of the concentrations used, but its maximum stimulation is

not reached until quantities much larger than are required of either the sulphate or the nitrate have been applied to the soil. While the carbonate stimulates throughout a much larger range of concentration than does any of the other manganese salts, it is not as powerful a stimulant as is the nitrate and especially the sulphate.

These results are intensely interesting when viewed in the light of results obtained by investigators who have studied the influence of manganese on higher plants. The majority of the investigators report the carbonate to be the greatest stimulant followed in the order, sulphate, nitrate and chloride. Furthermore, all workers have not found manganese compounds to stimulate the plants when applied directly to the soil, and the data herein reported offer a very plausible explanation of the lack of agreement among various reported experiments.

TABLE VIII
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING
2 GRAMS OF DRIED BLOOD TO WHICH WERE ADDED VARYING
AMOUNTS AND KINDS OF MANGANOUS SALTS

Fraction of Molecular Wgt. in 100 gm. Soil	Added Parts per Million Manganese—	Per cent of Ammonia Formed in Presence of			
		MnCl ₂	MnSO ₄	Mn(NO ₃) ₂	MnCO ₃
None	None	100.0	100.0	100.0	100.0
78x10 ⁻⁷	4.3	100.6	112.0	108.2	101.3
156x10 ⁻⁷	8.6	98.7	123.7	108.7	101.3
312x10 ⁻⁷	17.2	100.0	123.8	116.0	106.9
625x10 ⁻⁷	34.3	100.6	112.5	112.6	110.8
125x10 ⁻⁶	68.6	91.3	98.4	108.2	108.7
25x10 ⁻⁵	137.3	85.3	90.6	104.9	110.2
5x10 ⁻⁴	274.6	82.9	85.6	93.9	111.2
1x10 ⁻³	549.6	70.6	78.6	76.0	102.5
2x10 ⁻³	1098.2	60.5	77.8	63.3	106.9
3x10 ⁻³	1648.8	53.9	77.8	43.7	104.4
4x10 ⁻³	2198.4	51.2	63.4	38.4	104.9
5x10 ⁻³	2748.0	35.5	58.3	36.4	102.9
6x10 ⁻³	3297.6	35.5	47.5	27.5	100.0
7x10 ⁻³	3847.2	31.1	42.8	24.8	97.6
8x10 ⁻³	4396.8	28.7	39.2	18.7	100.0
9x10 ⁻³	4905.4	23.7	36.2	16.3	104.0
10x10 ⁻³	5496.0	14.7	43.2	13.4	101.3
11x10 ⁻³	6045.6	13.4	30.8	9.8	97.6

If we admit that much of the beneficial effect of the manganese on the plant is due to its stimulating influence on the bacterial activity of the soil, thus liberating more available nitrogen, forming organic acids and carbon dioxide which in turn liberate phosphorus and other elements essential to plant growth, we can readily see that its influence upon a plant growing in a soil well supplied with available nitrogen and phosphorus would not be great. But if the soil contained unavailable plant-food, the increased bacterial activity would make more plant-food available. This would then be taken up by the growing plant and shown in the increased crop yield.

The chloride and the sulphate of manganese both became toxic when equivalent molecular proportions of the salts were applied to the soil, but four times as much of the nitrate as of the sulphate can be applied to a soil before it becomes toxic. The toxicity of the nitrate increases much more rapidly than does the toxicity of the chloride or sulphate. When 4×10^{-3} mol. of manganous chloride is applied to 100 gm. of soil, it reduces the ammonifying powers of the soil to 51.2 per cent, while an equivalent quantity of the sulphate reduces it to 63.4 per cent. The nitrate in this concentration reduces it to 38.4 per cent, but the carbonate is still acting as a stimulant. The highest concentration tested, 11×10^{-3} mol., practically stops ammonification in the presence of the nitrate and chloride, while the sulphate reduces it to one-third its original, but at this concentration the carbonate is without effect. In low concentrations the chloride is the most toxic of the salts tested while in higher concentrations the nitrate is the more toxic.

The Influence of Iron Salts

The compounds used in this series were ferric chloride, ferric sulphate, ferric nitrate and ferrous carbonate. All except the carbonate were added to the soil in solution. The carbonate was added in the form of a dry powder and carefully mixed with the soil. Considerable difficulty was experienced in getting duplicate determinations to agree when the sulphate was applied to the soil and the results as reported for sulphate represent the average of eight sets of determinations. The chloride, nitrate, and carbonate represent the average of four closely agreeing sets of determinations. The results are given in Table IX.

From these results it may be seen that all of the iron salts tested act as stimulants to the ammonifying organisms of the soil. The maximum stimulation for the chloride occurs when 2.9 parts per million of iron have been applied to the soil, while in the case of the other three compounds the greatest stimulation is not noticed until twice this quantity of iron in the various forms has been applied. Furthermore, the chloride is a much more powerful stimulant than is the sulphate, and in this respect there is a marked difference between the salt of manganese and that of iron.

The results offer a very likely explanation of why there is an increased yield obtained when iron compounds are applied to the soil, as the stimulation of the soil organisms would greatly increase the available plant-food. There would be not only more available nitrogen, but the increased bacterial activity would render soluble more potassium and especially more phosphorus; the results reported by Griffiths (15) indicate that the plants growing on soil manured with iron sulphate contain more phosphorus than do those growing on unmanured soil. We would have to assume either that the application of iron to the soil stimulates a plant

so that it requires more phosphorus, or else that the iron compounds increase the availability of the phosphorus, and hence the plant takes up more. This latter explanation seems the more reasonable, but here we have to look for an indirect effect, for the iron directly depresses the solubility of phosphorus (14).

The ferric sulphate becomes toxic to the ammonifying organisms at a much lower concentration than do the other iron compounds, but it does not increase in toxicity nearly as rapidly as either the chloride or the nitrate. When 1489.2 parts per million of iron in the form of iron chloride or nitrate have been applied to a soil, its ammonifying powers are reduced to one-half their original, while an equivalent quantity of iron in the form of the sulphate reduces the ammonifying power to only 77.7 per cent. The carbonate, on the other hand, reduces it only 1.4 per cent.

TABLE IX
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING
2 GRAMS OF DRIED BLOOD AND TO WHICH HAD BEEN ADDED VARY-
ING AMOUNTS AND DIFFERENT FORMS OF IRON

Iron—Parts per Million Added	Per cent of Ammonia Formed in the Presence of			
	FeCl ₃	Fe ₂ (SO ₄) ₃	Fe(NO ₃) ₃	FeCO ₃
None	100.0	100.0	100.0	100.0
2.9	118.6	102.2	102.5	100.7
5.8	108.9	103.9	102.9	107.9
11.6	108.5	96.5	102.8	105.1
23.2	100.5	86.9	100.9	101.0
46.5	92.9	84.0	100.1	102.0
93.0	81.4	84.2	94.8	101.0
186.0	72.3	84.3	94.8	102.1
372.3	57.9	84.9	78.3	98.3
744.6	52.4	78.9	76.1	98.3
1116.9	52.1	79.7	67.1	99.0
1489.2	46.7	77.7	50.5	98.6
1861.5	40.6	73.9	44.5	96.3
2233.8	35.8	72.0	44.4	92.5
2606.1	36.2	78.7	44.5	93.5
2978.4	27.1	85.3	44.9	92.9
3350.7	25.7	72.3	44.1	92.2
3723.0	24.9	71.6	43.6	90.8
4095.3	17.7	76.8	30.3	87.8

The highest concentration used, 4095.3 parts per million of iron in the form of the chloride, reduces the ammonifying powers to 17.7 per cent, the nitrate to 30.3 per cent, the sulphate to 76.8 per cent, and the carbonate to 87.8 per cent.

The Influence of Chlorides

So far in this discussion we have been comparing the action of compounds having the same electro-positive but a varying electro-negative ion. Hence, the results considered have given us an insight into the influence of the anions Cl, SO₄, NO₃, and CO₃ upon the ammonifying efficiency of the soil. It is therefore important that the compounds be

compared where the anion is a constant and the cation a variable. This is done in Table X. In this series we have the chlorides of sodium, potassium, magnesium calcium, manganese, and iron. The experiment was so arranged that equivalent quantities of chlorine in the various forms were applied to 100 gm. of soil. Each reported result is the average of four or more closely agreeing determinations.

TABLE X
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD TO WHICH WAS ADDED VARYING AMOUNTS AND KINDS OF CHLORIDES, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

Cl—Parts per Million	Per cent of Ammonia Formed in the Presence of					
	NaCl	KCl	MgCl ₂	CaCl ₂	MnCl ₂	Fe ₂ Cl ₆
None	100.0	100.0	100.0	100.0	100.0	100.0
5.50	106.0	94.6	100.7	95.7	100.7	118.6
11.05	106.1	95.8	99.0	94.9	98.7	108.9
22.10	97.9	91.9	98.0	87.6	100.0	108.5
44.30	92.4	89.1	96.6	86.7	100.7	100.5
88.65	88.5	85.9	95.7	83.0	91.3	92.9
177.30	73.4	80.5	95.6	70.6	85.3	81.4
354.60	67.3	76.3	86.9	59.7	82.9	72.3
709.20	57.6	64.2	74.9	59.4	70.6	57.9
1418.40	40.6	50.5	64.2	41.8	60.5	52.4
2127.60	29.9	42.7	64.2	38.5	53.9	52.1
2836.80	24.1	38.6	57.6	34.2	51.2	46.7
3546.00	17.9	27.9	41.7	32.1	35.5	40.6
4255.20	17.9	22.3	41.5	32.1	35.5	35.8
4964.40	16.2	22.3	37.6	25.4	31.1	36.2
5673.60	16.1	18.4	36.3	24.2	28.8	27.1
6382.80	11.8	15.6	28.8	23.3	23.7	25.7
7092.00	11.5	14.8	19.3	19.7	14.7	24.9
7801.20	11.2	14.8	15.2	19.1	13.4	17.7

All of the chlorides tested, with two exceptions—calcium chloride and potassium chloride—increase the accumulation of ammonia in the soil, and it would appear from these results that the extent of stimulation is governed largely by the cation, while the toxicity of the compound is determined by the anion. Measured in terms of the effect upon ammonification, ferric chloride is the most effective stimulant of the chlorides tested, followed in the order of sodium chloride, magnesium chloride and manganous chloride. The last two stimulate only slightly. When 88.65 parts per million of chlorine in the form of iron or of manganese are applied to a soil they are toxic to the ammonifying organisms, while one-fourth this quantity of chlorine in the form of sodium is toxic and only one-half as much chlorine in the form of magnesium as in the form of sodium is necessary to decrease bacterial activity. Potassium chloride and magnesium chloride are toxic when only 5.5 parts per million are applied to a soil. If these two compounds act as stimulants it must be in very dilute solutions. Apparently the compounds, the cation of which has the highest atomic weight, are greater stimulants than those with

lower atomic weights. Those compounds, which in dilute solution act as stimulants, in more concentrated solutions increase more rapidly in toxicity than do compounds which are not as active stimulants.

At the highest concentration tested, 7801.2 parts per million of chlorine, there is only 8 per cent more ammonia found in the presence of the least toxic than in the presence of the most toxic compound. However, at this high concentration the soil produces less than 20 per cent of the quantity of ammonia produced in untreated soil. The least is produced in the presence of sodium chloride and the greatest in the presence of calcium chloride. But these two compounds come very nearly together when they are compared from the viewpoint of equal molecular proportions of the various salts, as is done in Table XI.

TABLE XI
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING
2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING
AMOUNTS AND KINDS OF CHLORIDES IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Wgt. in 100 gm. Soil	Per cent of Ammonia Formed in the Presence of				
	NaCl	KCl	MgCl ₂	CaCl ₂	MnCl ₂
None	100.0	100.0	100.0	100.0	100.0
156x10 ⁻⁷	106.0	94.6	98.8	94.9	98.7
312x10 ⁻⁷	106.1	95.8	98.0	87.6	100.0
625x10 ⁻⁷	97.9	91.8	96.6	86.7	100.6
125x10 ⁻⁶	92.4	89.1	95.7	83.0	91.3
25x10 ⁻⁵	88.5	85.9	95.6	70.6	85.3
5x10 ⁻⁴	73.4	80.5	86.9	59.7	82.9
1x10 ⁻³	67.3	76.3	74.9	59.4	70.6
2x10 ⁻³	57.6	64.2	64.2	41.8	60.5
4x10 ⁻³	40.6	50.5	57.6	34.2	51.2
6x10 ⁻³	29.9	42.7	41.5	32.1	35.5
8x10 ⁻³	24.1	38.6	36.3	24.2	28.7
10x10 ⁻³	18.0	27.9	19.3	19.7	14.7

When 312x10⁻⁷ mol. of sodium chloride is applied to 100 gm. of soil it acts as a stimulant to the ammonifying organisms, but an equivalent quantity of any of the other salts tested is toxic. Were it not for the potassium salt which would have about the same osmotic pressure as the sodium, one might attribute the toxicity to the greater osmotic pressure exerted by the other salts; but the results show that while the increased osmotic pressure undoubtedly plays considerable part in retarding the ammonifying organisms; yet the nature of the salt also must be taken into account. With the single exception of sodium chloride the quantity of ammonia obtained is less when 156x10⁻⁷mol. of the various salts are applied to the soil than when no salts are applied. At the highest concentration tested, 10x10⁻³ mol. per 100 gm. of soil, the potassium salt is least toxic, while the manganese salts are the most toxic. However, at this high concentration we find the quantity of ammonia produced in each very low and not a great difference between those receiving the various salts.

Influence of Sulphates

The compounds used in this series were the sulphates of potassium, sodium, calcium, magnesium, manganese and iron. The quantity of the salt used in each case was such that equivalent quantities of SO_4 in the various forms were added to 100 gm. of soil. It was also of such a concentration, with the exception of the iron sulphate, that equal molecular proportions of the various salts were added to 100 gm. of soil. The results as averages of a number of closely agreeing determinations are given in Table XII.

TABLE XII
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING AMOUNTS AND FORMS OF SULPHATES, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

Fraction of Molecular Wgt. in 100 gm. Soil	SO_4 —Parts per Million	Per cent of Ammonia Formed in the Presence of					
		Na_2SO_4	K_2SO_4	CaSO_4	MgSO_4	MnSO_4	$\text{Fe}_2(\text{SO}_4)_3$
None	None	100.0	100.0	100.0	100.0	100.0	100.0
78×10^{-1}	7.5	96.0	99.6	100.3	99.9	112.0	102.2
156×10^{-1}	15.0	95.2	98.4	100.6	98.7	123.7	103.9
312×10^{-1}	30.0	100.0	98.4	102.6	101.3	123.8	96.5
625×10^{-1}	60.0	97.6	98.9	103.2	104.3	112.5	86.9
125×10^{-2}	120.1	96.8	90.9	103.5	97.8	98.4	84.0
25×10^{-2}	240.2	96.0	87.0	102.0	94.4	90.6	84.2
5×10^{-2}	480.4	92.2	86.0	103.2	92.3	85.6	84.3
1×10^{-2}	960.8	86.5	74.1	98.8	84.0	78.6	81.9
2×10^{-3}	1921.5	72.4	56.6	98.0	82.8	77.8	78.9
3×10^{-3}	2882.3	66.8	53.7	96.6	82.8	77.8	79.7
4×10^{-3}	3843.1	62.8	53.7	98.8	77.9	63.4	77.7
5×10^{-3}	4803.8	55.2	51.8	91.0	77.3	58.3	73.9
6×10^{-3}	5764.6	51.6	53.7	93.6	77.2	47.5	72.0
7×10^{-3}	6725.5	46.0	51.5	97.4	70.7	42.8	78.7
8×10^{-3}	7686.1	42.8	50.8	95.7	51.3	39.2	85.3
9×10^{-3}	8647.0	40.5	41.8	96.0	48.4	36.2	72.3
10×10^{-3}	9607.7	33.2	37.5	95.1	43.6	34.2	71.6
11×10^{-3}	10568.6	32.0	30.7	96.5	36.2	30.8	76.8

Sodium sulphate and potassium sulphate are the only sulphates which fail to stimulate the ammonifying organisms. The intensity of the stimulation and the degree of stimulation varies with the different compounds. Calcium sulphate and manganous sulphate, which are so widely recognized as plant stimulants, have the greatest effect also upon the bacterial activity. It is quite likely that their influence upon the plant is only a secondary effect due to the action of the compound upon the soil organisms, which in turn liberate more plant-food. It is interesting to note that calcium sulphate, which is so widely known as a soil stimulant, increases the ammonia produced in the soil at first, but when 1×10^{-3} mol. or more is applied to 100 gm. of soil, it becomes slightly toxic. The toxicity, however, does not greatly increase as more of the salt is applied for we find 96.5 per cent of ammonia produced in the presence of 11×10^{-3} mol. The greater quantities of ammonia obtained in the lower concentrations of the salt cannot be attributed to the calcium sulphate

preventing volatilization of the ammonia formed. Otherwise, we should expect the stimulation to increase or at least to remain the same where larger quantities of calcium sulphate are applied to the soil.

The toxicity of potassium sulphate is perceptible in the lowest concentration tested and gradually increases as the salt applied increases, until at 8×10^{-3} mol. the ammonifying power of the soil had been reduced to 50.8 per cent, and at the highest concentration tested, 11×10^{-3} mol. per 100 gm. of soil, it had been reduced to 30.7 per cent.

The influence of sodium sulphate on the ammonifying efficiency of the soils is almost identical with that of potassium sulphate, showing that the toxicity of the salt is governed largely by the electro-negative ion.

Magnesium sulphate and manganous sulphate differ from the sodium and potassium sulphates in that the former does not become toxic until 125×10^{-6} mol. of the salt has been applied to 100 gm. of the soil, but above this concentration the toxicity of these compounds increases more rapidly than does the toxicity of sodium sulphate or potassium sulphate. In the highest concentration tested, 11×10^{-3} mol., it is interesting to note that the toxicity of all the readily soluble salts is the same. From the reported data, it would appear that ferric sulphate is an exception to this statement, but it must be borne in mind that while the quantity of sulphate in this is the same as in the other sulphates yet the number of molecules of the salt is less. Furthermore, it would appear that the iron ion has actually had a protective influence against the toxic SO_4 ion. Moreover, ferric sulphate has a great deflocculating effect upon the soil, for after its addition the soil remains very light and porous.

The Influence of Nitrates

The compounds used in this series were sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, manganous nitrate and ferric nitrate. The quantity added to the soil was such that in each case equivalent quantities of NO_3 in the various forms were added to the soil. Hence, the varying factor is the electro-positive ion, the electro-negative remaining the same in each case. The average results for a number of closely agreeing determinations are given in Table XIII as percentages of ammonia formed in 100 gm. of soil, the untreated soil being taken as 100 per cent.

Sodium nitrate in the lower concentrations stimulates the ammonifying organisms of the soil and not until the quantity added reaches 310.4 parts per million of NO_3 does the compound become toxic. When 6208 parts per million have been added to the soil the ammonifying efficiency of the soil is reduced to less than half normal. At the highest concentration tested, 13,657.6 parts per million, the ammonia produced was still 35.4 per cent of its original.

Potassium nitrate is not as strong a stimulant as is the sodium nitrate and becomes toxic in lower concentration than does the sodium nitrate.

However, the results as a whole show considerable similarity between the sodium and potassium compounds. At the highest concentration used potassium nitrate is considerably more toxic to ammonifying organisms than is sodium nitrate.

Calcium nitrate and magnesium nitrate are very similar in their action on the ammonifying powers of the soil. Neither stimulates in any of the concentrations tested, and they run nearly parallel throughout the entire range of concentration used. Calcium nitrate is slightly more toxic than is the magnesium nitrate. It is interesting to note the very small quantities of these substances required to depress the accumulation of ammonia in the soil. While they become toxic in very dilute solutions their toxicity does not increase as rapidly as does the toxicity of sodium nitrate and especially of potassium nitrate.

TABLE XIII
PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD TO WHICH WAS ADDED VARYING AMOUNTS AND FORMS OF NITRATE, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

NO ₃ —Parts per Million	NaNO ₃	KNO ₃	Per cent of Ammonia Formed in the Presence of			
			Ca(NO ₃) ₂	Mg(NO ₃) ₂	Mn(NO ₃) ₂	Fe(NO ₃) ₃
None	100.0	100.0	100.0	100.0	100.0	100.0
9.7	104.6	102.2	91.2	95.6	108.2	102.5
19.4	103.7	100.4	91.0	90.7	108.7	102.9
38.8	102.3	101.8	90.0	88.3	116.0	102.8
77.6	104.4	99.1	87.1	82.3	112.6	100.9
155.2	107.8	98.3	86.2	80.0	108.2	100.1
310.4	94.2	95.0	81.0	92.0	104.9	94.8
624.8	90.6	89.5	74.6	81.7	93.9	94.8
1241.6	81.7	83.5	66.0	83.0	76.0	78.3
2483.2	75.9	68.7	76.2	83.6	63.3	76.1
3724.8	56.0	50.7	75.8	75.1	43.7	67.1
4966.4	54.0	51.1	68.0	72.3	38.4	50.5
6208.0	46.6	55.4	60.4	69.5	36.4	44.5
7449.6	46.0	49.0	62.7	69.1	27.5	44.4
8691.2	41.5	41.6	61.8	57.9	24.8	44.5
9932.8	41.5	34.4	51.3	50.7	18.7	44.9
11174.4	40.1	30.5	37.8	39.5	16.3	44.1
12416.0	37.9	26.5	27.9	33.8	13.4	43.6
13657.6	35.4	25.8	22.3	27.2	9.8	30.3

Manganous nitrate when applied to soil in small quantities is a more powerful stimulant of the ammonifying organisms of the soil than are any of the other nitrates tested, but when used in large quantities it is much more toxic than are any of the others. As a matter of fact, these results tend to bear out the general biological observation formulated by Hueppe that every substance, which in small quantities kills the protoplasm, in yet smaller quantities acts as a stimulant. Although manganous nitrate does not become toxic until 624.9 parts per million of the nitrate have been applied to the soil, in the highest concentration tested the ammonia produced is reduced to 9.8 per cent of normal, which is much lower than by any of the other nitrates.

Ferric nitrate, while it increases the ammonifying powers of the soil, is not as efficient in this respect as is the manganous nitrate. It does, however, become toxic in slightly lower concentrations than the manganous nitrate. But the toxicity of the latter increases much more rapidly than does that of the former, for at the highest concentration tested we find three times the ammonia produced in the presence of ferric nitrate as at an equivalent concentration of the manganous nitrate, pointing to the conclusion that the toxicity of these compounds is governed by both anion and cation. It would be wrong, however, to conclude that the Mn ion is so much more toxic to the ammonifiers than is the Fe ion, for it must be borne in mind that more manganese than iron has been applied to the soil.

If we arrange the compounds in their decreasing order of toxicity as measured at the highest concentration when equivalent quantities of nitrates are added to a soil, they are manganous nitrate, potassium nitrate, magnesium nitrate, ferric nitrate and sodium nitrate. If we compare them when equivalent molecular proportions of the salts are added, using the highest concentration, 10×10^{-3} mol. per 100 gm. of soil, the order is changed and becomes manganous nitrate most toxic followed in the order named by ferric nitrate, calcium nitrate, magnesium nitrate, sodium nitrate and potassium nitrate.

The Influence of Carbonates

The compounds used in this series were the carbonates of sodium, potassium, calcium, magnesium, manganese and iron. The results as percentages of ammonia, considering the untreated soil as 100 per cent, are given in Table XIV.

Sodium carbonate stimulates the ammonifying organisms of this soil in all concentrations tested up to and including 5×10^{-3} mol. per 100 gm. of soil, while potassium carbonate stimulates in concentrations below this. Hence, the potassium salt is more toxic than is the sodium salt. Furthermore, the extent of stimulation in the case of sodium carbonate is greater than it is when potassium carbonate is added to the soil. The point at which the two reach their maximum stimulation is practically the same. Even in the highest concentrations, 11×10^{-3} mol. per 100 gm. of soil, large quantities of ammonia are produced.

Neither calcium carbonate nor manganous carbonate were found to be toxic in any of the concentrations tested, and the stimulating influence of both compounds is very marked. The soil to which 8×10^{-3} mol. of calcium carbonate was added produced 114.6 per cent of ammonia, while the maximum stimulating influence of the manganous carbonate is reached at a lower concentration, 5×10^{-4} mol.; although a strong stimulant, it seldom increases the ammonifying powers to as great an extent as does calcium carbonate. Considerable of the influence exerted by both of these compounds must be due to their improving the physical condition of the soil.

For soils to which these are added never become hard and impervious to air and water as do soils to which potassium or sodium carbonate has been added.

The ferrous carbonate stimulates slightly until the quantity added exceeds 1×10^{-3} mol., but above this concentration, while it is toxic its toxicity never becomes great, most likely because of the comparative insolubility of the compound used.

TABLE XIV
PERCENTAGES OF AMMONIA FORMED IN 4 DAYS IN 100 GRAMS OF SOIL TO WHICH WAS ADDED 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND DIFFERENT FORMS OF CARBONATES

Fraction of Molecular Wgt. in 100 gm. Soil	Million Parts per CO ₂ —	Per cent of Ammonia Formed in the Presence of					
		Na ₂ CO ₃	K ₂ CO ₃	CaCO ₃	MgCO ₃	MnCO ₃	FeCO ₃
None	None	100.0	100.0	100.0	100.0	100.0	100.0
78×10^{-7}	4.7	105.6	103.8	97.3	100.5	100.3	100.7
156×10^{-7}	9.4	104.4	105.8	97.6	101.6	101.3	107.9
312×10^{-7}	18.7	106.8	107.7	101.2	103.4	106.9	105.1
625×10^{-7}	37.5	107.6	108.0	109.4	103.5	110.8	101.0
125×10^{-6}	75.0	107.6	106.1	108.4	101.0	108.7	102.0
25×10^{-5}	150.0	108.9	106.1	107.3	101.0	110.2	101.0
5×10^{-4}	300.0	110.1	106.1	108.7	95.6	111.2	102.1
1×10^{-3}	600.0	108.3	107.2	110.7	91.3	102.5	98.3
2×10^{-3}	1200.0	103.8	108.0	112.2	94.5	106.9	98.3
3×10^{-3}	1800.0	102.9	105.8	111.5	89.8	104.4	99.0
4×10^{-3}	2400.0	101.2	100.0	109.7	86.7	101.9	98.6
5×10^{-3}	3000.0	101.4	98.1	110.4	79.8	102.9	96.3
6×10^{-3}	3600.0	94.7	88.5	108.7	80.7	100.6	92.5
7×10^{-3}	4200.0	92.5	89.2	108.7	75.4	97.6	93.5
8×10^{-3}	4800.0	88.6	78.5	114.6	77.4	100.0	92.9
9×10^{-3}	5400.0	80.5	75.8	104.8	77.4	104.0	92.2
10×10^{-3}	6000.0	67.1	68.3	104.8	71.9	101.3	90.8
11×10^{-3}	6600.0	65.6	54.6	107.6	67.1	97.6	87.8

In the presence of 10×10^{-3} mol. of the various compounds per 100 gm. of soil, the average amount of ammonia produced with the chloride is 18.32 per cent. In the presence of an equivalent quantity of the nitrates it is 35.42 per cent. With the sulphate it is 47.72 per cent, and in the presence of the carbonate it is 84.03 per cent. Thus measured in terms of their effect upon the ammonifying efficiency of the soil, the chloride is the most toxic of the compounds tested, followed in order by nitrates, sulphates and carbonates.

Relationship between Ammonifying Organisms and Higher Plants

The results herein reported, together with those published by Dr. Harris (17) on "Effect of Alkali Salts in Soils on the Germination and Growth of Crops," make it possible to compare the influence of some of the salts upon the ammonifying powers of a soil with their influence upon the higher plants. This comparison is quite justifiable, for the same soil was used in the experiments with seedlings as has been used in this work on the ammonifiers. This comparison is made in Table XV.

The results as reported are the quantities of the respective salts which are necessary to reduce ammonia produced, germination of wheat and production of dry matter in wheat to about half normal.

The chlorides of sodium, calcium, potassium and magnesium rank in the order named in toxicity to ammonifiers, but in the case of the plant, sodium nitrate is less toxic than is magnesium chloride. The quantities of the various chlorides which are necessary to reduce plant growth and ammonia to half normal were found to be very nearly the same. The order of toxicity of the salts, with the exception of sodium nitrate, sodium carbonate, magnesium nitrate and potassium sulphate is the same for both the wheat seedlings and the ammonifying organisms.

TABLE XV
PERCENTAGES OF VARIOUS SALTS IN LOAM SOIL WHICH ARE NECESSARY TO
REDUCE AMMONIFICATION, GERMINATION AND DRY MATTER
PRODUCED IN WHEAT TO ABOUT HALF NORMAL

	Ammonification to Half Normal	Wheat Seedling to Half Normal	Excess Required by Bacteria
Sodium Chloride	0.117	.20	.083
Calcium Chloride	0.222	.30	.078
Potassium Chloride	0.298	.25	.048
Magnesium Chloride	0.381	.40	.019
Potassium Nitrate	0.607	.40	.207
Sodium Nitrate	0.850	.30	.550
Sodium Sulphate	0.852	.55	.302
Magnesium Sulphate	0.963	.70	.263
Sodium Carbonate	1.166	.30	.866
Magnesium Nitrate	1.187	.45	.737
Potassium Sulphate	1.394	.60	.794
Potassium Carbonate	1.520	.70	.820

The other compounds are more toxic for the wheat seedlings than they are for ammonifying organisms. The greatest difference is in the case of sodium carbonate which was found to be very toxic to plants, but the bacteria withstand large quantities. Lipman (27) found ammonifying organisms to withstand even larger quantities than herein reported, for according to him it requires between 1.8 and 2 per cent of sodium carbonate to reduce the ammonia production to half normal.

Most of the salts tested are more toxic to wheat seedlings than they are toward the ammonifying organisms. This is very marked in the case of the carbonates, nitrates and sulphates, for it requires nearly twice the quantity of these to reduce ammonia produced to one-half normal as is required to reduce wheat seedling growth to the same extent.

The Relative Stimulation of the Various Salts

It has been noted repeatedly throughout this work that many of the salts tested stimulate the ammonifying organisms. The extent of stimulation and the concentration of the specific salts required to produce maximum stimulation vary greatly with the salt. These facts are summarized in figure 1.

Only six of the compounds tested—calcium chloride, calcium nitrate, potassium chloride, potassium sulphate, magnesium nitrate and sodium sulphate—failed to stimulate in any of the concentrations. The other eighteen salts all stimulated the ammonifying organisms of the soil. Among these, however, there is a great variation in the extent of the stimulation. It is significant that we find as the more active stimulants those compounds which are most active in stimulating plant growth.

It is likely that the stimulation of the plant is due to a great extent to the increased available plant-food yielded by the increased bacterial activity. Averaging the molecular weights for the twelve compounds acting as the strongest stimulants, we find them to be slightly higher than the average molecular weight of those which exert little stimulating influence. But, comparing individual stimulants and non-stimulants we find no relationship to exist, for sodium chloride with the lowest molecular weight is a strong stimulant, while potassium sulphate with a high molecular weight is devoid of stimulating influence. Hence, it would seem that Grützner's generalization for animal stimulants will not hold for the ammonifying organisms of the soil.

The quantity of the salt necessary to produce maximum stimulation varies greatly with the salt. The iron compounds all stimulate most in low concentrations, while the carbonates of calcium, manganese and sodium have to be added in large quantities to a soil before maximum stimulation is noted. It is interesting to note that of the various salts studied by Miyake (41) and found by him to stimulate the rice plant, calcium chloride is the only one which stimulated the rice that does not stimulate the ammonifying organisms of the soil. Furthermore, Dr. Harris (17) found that sodium chloride, potassium sulphate, magnesium nitrate, calcium chloride and magnesium chloride failed to stimulate wheat seedlings, while sodium nitrate, sodium sulphate, magnesium sulphate, potassium nitrate, potassium chloride, potassium sulphate and sodium carbonate all acted as stimulants. Of these compounds sodium carbonate is the most active stimulant which is similar to the findings for the ammonifying efficiency of the soil.

Relative Toxicity of the Various Salts

The salts used in this work may be compared as to toxicity from three viewpoints: first, the lowest concentration of the salt at which a toxic effect is noted toward the ammonifying organisms; second, the molecular concentration at which ammonia produced is reduced to three-fourths normal; and third, the per cent of ammonia produced in the presence of the largest quantity of the various salts, which is 10×10^{-7} mol. of the salt in 100 gm. of soil. These results are given in figures 2, 3 and 4.

Five of the compounds tested—calcium nitrate, magnesium nitrate, calcium chloride, sodium sulphate and potassium sulphate—were toxic to

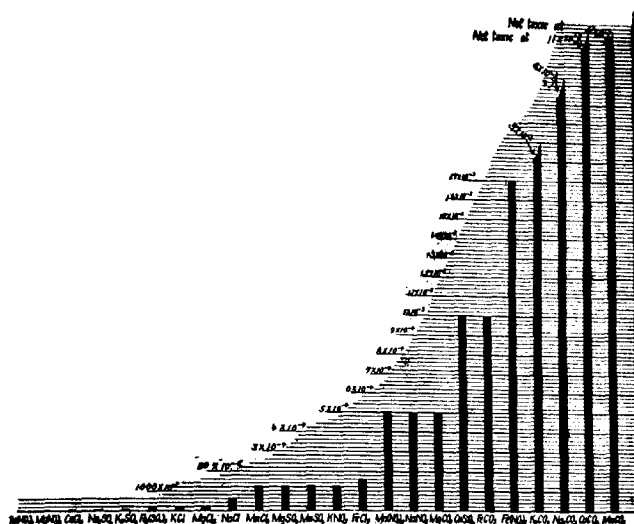


Fig. 2.—Diagram showing the molecular concentrations at which the various salts are toxic to ammonification.

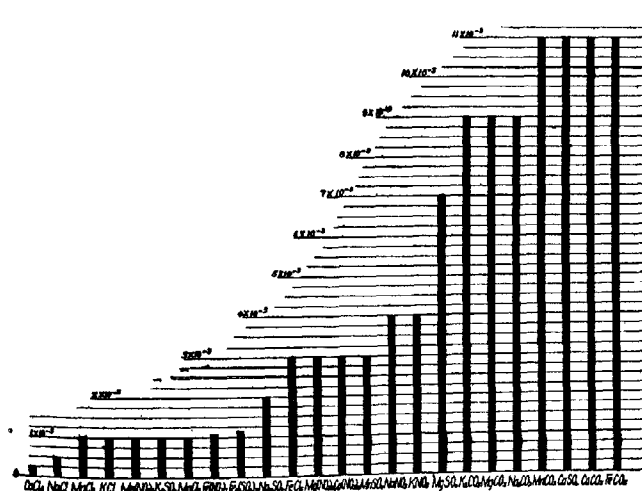


Fig. 3.—Diagram showing the molecular concentrations which reduce the ammonia produced to three-fourths normal.

the ammonifiers in the lowest concentration tested, which was 78×10^{-3} mol. per 100 gm. of soil. All of the others, with the exception of two—calcium carbonate and manganous carbonate—became toxic in some of the concentrations tested. It is quite evident from these results that while the increased osmotic pressure exerted by the salts added to a soil plays an important part in the retarding of the bacterial activity, it is not the only nor probably the main influence. The main influence is likely to be a physiological one, due to the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it cannot function normally. However, we do not find a relationship between the toxicity of the compound and its power to precipitate colloids. It appears, therefore, that while the precipitation of the colloidal cellular material often causes death of the organisms, it is not necessarily the determining factor in the toxic action of these salts.

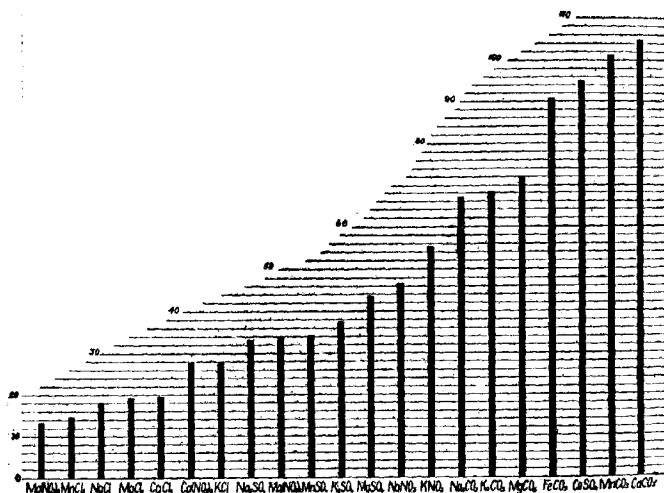


Fig. 4.—Diagram showing the percentages of ammonia produced in 100 gm. of soil to which had been added 10×10^{-3} molecules of the various salts. untreated soil being counted as producing 100 per cent.

As can be seen from figure 3 it is not necessarily those compounds which become toxic at the lowest concentration which have the most far-reaching effect upon the bacterial activity. For we find many of the compounds which were toxic at the lowest concentration tested must be added in fairly large quantities before the ammonia produced is reduced to three-fourths normal. For example, we find sodium sulphate toxic in 78×10^{-7} mol. concentration, yet it does not reduce the ammonia produced to three-fourths normal until 2×10^{-3} mol. concentration has been reached.

While manganous chloride does not become toxic until 125×10^{-6} mol. has been added to 100 gm. of soil, yet at a concentration of 1×10^{-3} mol. it has reduced the ammonia production to three-fourths normal. From the results as a whole it can be seen that the common soil alkalies—sodium chloride, calcium chloride, sodium sulphate—and the less common one—calcium nitrate—are very toxic to ammonifying organisms, and if present to any great extent will greatly reduce the ammonia produced in the soil.

An examination of figure 4 shows conclusively that the toxicity of the compounds is controlled largely by the cation, for we find invariably at the concentration 10×10^{-3} mol. the chlorides and nitrates very greatly reduced the ammonifying powers of the soil, while the carbonates only slightly reduced the ammonia. The extent to which the carbonate reduces the ammonifying efficiency of the soil is nearly proportional to the solubility of the compound.

SUMMARY

The toxicity of the chlorides, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron as determined by ammonification is controlled largely by the electro-negative ion and as a general rule, to which there are exceptions, the chlorides are the most toxic and nitrates, sulphates, and carbonates follow in the order of decreasing toxicity.

The quantity of a salt which can be applied to a soil without decreasing the ammonia formed varies with the salt and for the soil under investigation it is in the order of decreasing toxicity of the salts as follows: calcium chloride, calcium nitrate, sodium sulphate, potassium sulphate, magnesium nitrate, ferric sulphate, potassium chloride, magnesium chloride, sodium chloride, manganous chloride, magnesium sulphate, manganous sulphate, potassium nitrate, ferric chloride, manganous nitrate, sodium nitrate, magnesium carbonate, calcium sulphate, ferrous carbonate, ferric nitrate, potassium carbonate, sodium carbonate, manganous carbonate, and calcium carbonate. The last two were not toxic in any of the concentrations used.

It is not necessarily those compounds which become toxic in the lowest concentrations which are most toxic in higher concentrations; as the toxicity of some salts increases more rapidly than the toxicity of others.

It is quite evident from the results reported that the increased osmotic pressure exerted by the salt added to the soil plays a great part in the retarding of the bacterial activity but it is not the only factor. The main factor is probably a physiological one due to the action of the substance upon the living protoplasm of the cell changing its chemical and physical properties so that it cannot function normally.

The common soil "alkalies," sodium chloride, calcium chloride, sodium sulphate,—and the less common one—calcium nitrate—are very toxic to

ammonifying organisms and if present in soil to any great extent will greatly reduce the ammonia produced in such a soil.

Calcium chloride, calcium nitrate, potassium chloride, potassium sulphate, magnesium nitrate, and sodium sulphate failed to increase the ammonia produced in a soil. All of the others, however, in some of the concentrations tested acted as stimulants. The extent of the stimulation and the quantity of salt necessary for maximum stimulation varied with the compound. Naming them in the order of increasing efficiency, they are: magnesium chloride, manganous chloride, potassium nitrate, ferric nitrate, magnesium carbonate, calcium sulphate, ferric sulphate, magnesium sulphate, sodium chloride, sodium nitrate, ferrous carbonate, potassium carbonate, sodium carbonate, manganous carbonate, calcium carbonate, manganous nitrate, ferric chloride, and manganous sulphate.

Those compounds which are most active as stimulants to the higher plants are also most active in stimulating bacteria, and it is very likely that the effect upon the plant is due to the action of the compound upon the bacteria, which in turn render available more plant-food.

The quantity of sodium chloride, calcium chloride, potassium chloride and magnesium chloride required to reduce the ammonifying powers of the soil to half normal is practically the same as the quantity necessary to reduce the growth of wheat to the same extent. The ammonifying organisms are apparently more resistant to the other compounds tested than are the higher plants.

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ON THE NATURE OF AMMONIFICATION AND NITRIFICATION¹

By

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It has been found by many experiments on ammonification and nitrification that the rates of increase of ammonia and nitric acid produced at first rise very slowly, then very rapidly and later slowly again. If the rates are put in the form of a curve, the curve is very similar to that of an autocatalytic chemical reaction in which one of the products of reaction accelerates the reaction. It will be of interest, therefore, to study the nature of these microbiological chemical phenomena from the standpoint of autocatalysis.

THE QUANTITATIVE RELATION BETWEEN THE AMOUNT OF REACTION-PRODUCTS AND THE TIME OF AUTOCATALYSIS

The differential equation which is characteristic of the initial stages of autocatalytic mono-molecular reaction is as follows:

$$\frac{dx}{dt} = k_1 x (a - x) \dots \dots \dots (1)$$

which expresses in mathematical symbols the fact that the velocity of the transformation is, at any instant, proportional to the amount of material which is undergoing change and to the amount of material which has already been transformed. If, however, the reaction has proceeded so far that the depressant effect of the products of the reaction upon its velocity is measurable, equation (1) becomes:

$$\frac{dx}{dt} = k_1 x (a - x) - k_2 x^2 \dots \dots \dots (2)$$

This equation can, however, be reduced to the same form as (1), only the constants have a somewhat different meaning, for equation (2) is obviously the same as the following equation:

$$\frac{dx}{dt} = (k_1 + k_2)x \left(\frac{k_1}{k_1 + k_2} a - x \right) \dots \dots \dots (3)$$

which is of the same form as equation (1) but instead of k_1 we have $\frac{k_1}{k_1 + k_2}$, and instead of a we have $\frac{k_1}{k_1 + k_2} a$.

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Equation (3) may therefore be written:

$$\frac{dx}{dt} = kx(A - x) \dots\dots\dots (4)$$

where k and A are constants.

Integrating equation (4), we obtain:

$$\log \frac{x}{A - x} = Akt + C \dots\dots\dots (5)$$

where C is the constant of integration.

Now, it is evident that when $x = \frac{1}{2}A$, that is, when the reaction has

proceeded half way to equilibrium, $\log \frac{x}{A - x} = 0$. Choosing C , therefore, so as to make the right-hand side of equation (5) vanish at the same time as the left-hand side we have:

$$\log \frac{x}{A - x} = Ak(t - t_1) \dots\dots\dots (6)$$

where t_1 is the time at which the reaction is half completed. This equation may obviously be written,

$$\log \frac{x}{A - x} = K(t - t_1) \dots\dots\dots (7)$$

If an increase of reaction products with time can be regarded as expressing, primarily, the progress of an autocatalytic reaction, equation (7) should hold good. Where x represents the amount of reaction product at the time t , and A represents the maximum or final amount of reaction product, t_1 is the time at which half this maximum amount has been attained, and K is a constant which must be determined from a known value of x at a given time t .

On differentiating equation (4) with respect to x , calling $\frac{dx}{dt} = v$ we obtain:

$$\frac{dv}{dx} = k(A - 2x) \dots\dots\dots (8)$$

Differentiating again we obtain:

$$\frac{d^2v}{dx^2} = -2k \dots\dots\dots (9)$$

The right-hand side of equation (8) vanishes and $\frac{d^2v}{dx^2}$ is negative

when $x = \frac{A}{2}$, hence $\frac{dx}{dt}$ is at a maximum when $x = \frac{A}{2}$. In other words the rate of increase of reaction products during a given period of reaction should be at a maximum when the reaction of that period is half completed.

APPLICATION TO AMMONIFICATION

I.

Lipman (2), working in collaboration with Blair, Owen and McLean, has carried out a series of determinations upon the daily increase of ammonia formed in ammonification. In his experiment, 80 gm. of soil, 20 gm. of sand, 0.5 gm. of calcium carbonate and dried blood equivalent to 465 mg. of nitrogen were used. Six c.c. of soil infusion, 12 c.c. of water and 60 per cent of moisture for organic matter were added. The mixture was kept in tumblers at room temperature. The amounts of ammonia nitrogen recovered are indicated in the following table and the accompanying curve. On examining the table in which the daily increase of ammonia nitrogen is indicated, choosing the points with which to determine the constants of the curve sufficiently far apart and in such a manner that each pair of points on the curve is separated by an interval of 2 days, we find that:

When time = 2 days, Weight of ammonia nitrogen =	8.11 mg.
When time = 4 days, Weight of ammonia nitrogen =	46.03 mg.
When time = 8 days, Weight of ammonia nitrogen =	164.87 mg.
When time = 10 days, Weight of ammonia nitrogen =	185.91 mg.

Applying equation (7) to these values we find:

$$\log \frac{8.11}{A - 8.11} = K (2 - t_1) \quad (10)$$

$$\log \frac{46.03}{A - 46.03} = K (4 - t_1) \quad (11)$$

$$\log \frac{164.17}{A - 164.17} = K (8 - t_1) \quad (12)$$

$$\log \frac{185.91}{A - 185.91} = K (10 - t_1) \quad (13)$$

From (10) and (11), by subtraction we obtain:

$$\log \frac{46.03 (A - 8.11)}{8.11 (A - 46.03)} = 2K \quad (14)$$

While from (12) and (13) we obtain:

$$\log \frac{185.91 (A - 164.17)}{164.17 (A - 185.91)} = 2K \dots\dots\dots (15)$$

Hence, from (14) and (15) we have:

$$\frac{46.03 (A - 8.11)}{8.11 (A - 46.03)} = \frac{185.91 (A - 164.17)}{164.17 (A - 185.91)} \dots\dots\dots (16)$$

Solving this equation for A we find:

$$A = 189.83$$

A convenient check upon the above calculation is afforded by the fact expressed in equations (8) and (9), namely that the rate of increase in x , that is, daily increase in weight, should be at a maximum when x

$$(\text{= amount of ammonia nitrogen}) = \frac{1}{2} A, \text{ in this case } 94.94 \text{ mg.}$$

Examining the table we find that:

At time = 3 days, Weight of ammonia nitrogen = 19.80 mg.
Daily increase = 11.70 mg.

At time = 4 days, Weight of ammonia nitrogen = 46.03 mg.
Daily increase = 26.23 mg.

At time = 5 days, Weight of ammonia nitrogen = 68.66 mg.
Daily increase = 22.63 mg.

At time = 6 days, Weight of ammonia nitrogen = 123.23 mg.
Daily increase = 54.57 mg.

At time = 7 days, Weight of ammonia nitrogen = 144.27 mg.
Daily increase = 21.04 mg.

At time = 8 days, Weight of ammonia nitrogen = 164.87 mg.
Daily increase = 20.60 mg.

Thus we see that when the amount of ammonia nitrogen is between 68.66 and 123.23 mg., the rate of increase in ammonia nitrogen is at a maximum. Hence we may conclude that the value of A deduced from equation (16) is the correct one.

Now we find from the curve that the amount of ammonia nitrogen is 94.94 mg. at 5.6 days. Hence equation (7) becomes for this case:

$$\log \frac{x}{189.83 - x} = K (t - 5.6) \dots\dots\dots (17)$$

Now, when $t = 1$ we have:

$$\log \frac{5.56}{184.27} = -4.6 K$$

$$K = 0.3305$$

Similarly:

$t = 2$	$K = 0.3753$
$t = 4$	$K = 0.3100$
$t = 5$	$K = 0.4097$
$t = 8$	$K = 0.3415$
$t = 10$	$K = 0.3810$

The average value of K from these values is 0.358, hence equation (7) becomes:

$$\log \frac{x}{189.83 - x} = 0.358 (t - 5.6) \dots \dots \dots (18)$$

By giving t the successive value 1, 2, 3, etc., we can calculate the corresponding theoretical value of x , that is, of the weight of ammonia nitrogen. In Table I and figure 1 the theoretical and experimental results are compared.

TABLE I
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	5.55	4.19
2	8.11	9.29
3	19.80	19.94
4	46.03	40.10
5	68.66	72.01
6	123.24	110.55
7	144.27	144.78
8	164.87	166.99
9	172.70	179.23
10	185.91	185.20

It will be seen that in nearly every case the observed and calculated values agree well.

II.

Applying the same method to another observation of Lipman's upon the daily increase of ammonia nitrogen in ammonification in which dried blood equivalent to 310 mg. of nitrogen was added, and using the same points ($t = 2, 4, 8$ and 10) on the curve to determine A , we find:

$$A = 133.13$$

Hence $\frac{1}{2} - A = 66.57$. Now referring to Table II we find that:

At time = 3 days, Weight of ammonia nitrogen = 15.14 mg.

Daily increase = 9.06 mg.

At time = 4 days, Weight of ammonia nitrogen = 31.07 mg.

Daily increase = 15.93 mg.

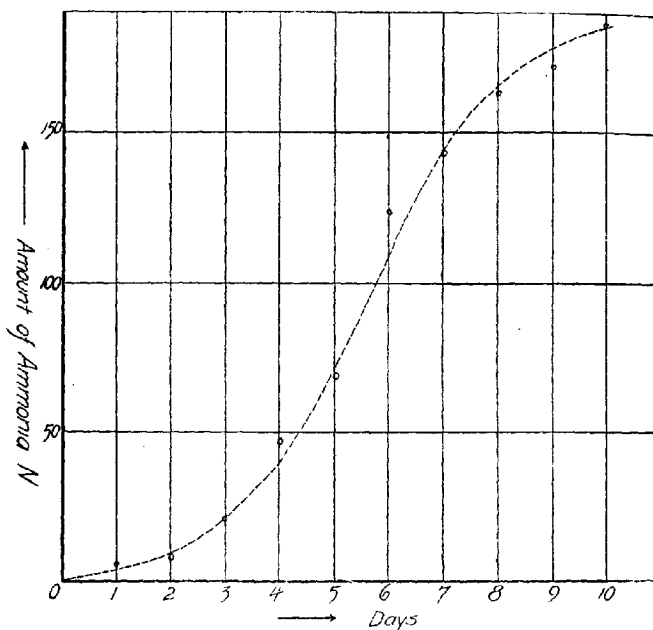


Fig. 1.—Diagram showing a comparison of the amount of ammonia nitrogen found with that calculated by formula.

At time = 5 days, Weight of ammonia nitrogen = 51.14 mg.

Daily increase = 20.07 mg.

At times = 6 days, Weight of ammonia nitrogen = 81.07 mg.

Daily increase = 29.93 mg.

At time = 7 days, Weight of ammonia nitrogen = 98.32 mg.

Daily increase = 17.25 mg.

At time = 8 days, Weight of ammonia nitrogen = 108.27 mg.

Daily increase = 9.95 mg.

Hence the agreement between the theoretical and actual curves of increase of ammonia nitrogen is very good, for when $x = \frac{1}{2} A = 66.57$ the daily increment has reached its maximum.

On the curve we find 5.6 days corresponding to 66.57 mg. of ammonia nitrogen. Hence the equation becomes for this case:

$$\log \frac{x}{133.13 - x} = K (t - 5.6)$$

From all of the observed value we find that the average value of K is 0.3352, hence the equation becomes:

$$\log \frac{x}{133.13 - x} = 0.3352 (t - 5.6)$$

As before, giving the successive value 1, 2, 3, etc. for t , we can compute the corresponding theoretical value of x . In Table II the theoretical and observed results are compared.

TABLE II
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	4.24	3.72
2	6.08	7.78
3	15.14	15.77
4	31.07	30.00
5	51.14	51.41
6	81.07	76.75
7	98.32	99.39
8	108.27	115.06
9	119.36	124.14
10	128.51	128.78

III

Applying the same method to another experiment of Lipman's in which dried blood equivalent to 155 mg. of nitrogen was added and using the points ($t = 3, 5, 6$ and 8) on the curve to determine A , we find:

$$A = 82.10$$

$$\frac{A}{2} = 41.05$$

The daily increment of ammonia nitrogen is at a maximum between 25.52 and 54.04 mg. of ammonia nitrogen. Hence the value of A deduced from the equation is the correct one. The time to produce 41.05

mg. of ammonia nitrogen is 5.6 days. Hence the equation becomes for this case:

$$\log \frac{x}{82.10 - x} = K (t - 5.6)$$

By calculating the value of K from the observations at $t = 1, 2, 3, 4, 7$ and 8 we find, respectively, $0.2927, 0.3461, 0.3698, 0.4134, 0.3889$ and 0.3781 . The average of these values is 0.3651 . Hence the equation becomes:

$$\log \frac{x}{82.10 - x} = 0.3651 (t - 5.6)$$

TABLE III
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	3.34	1.68
2	4.41	3.79
3	8.13	8.21
4	14.70	16.96
5	25.52	30.91
6	54.04	47.90
7	63.73	62.76
8	73.06	72.44
9	55.10	77.10
10	61.53	80.10

Now giving t the successive value $1, 2, 3$, etc., we find the following theoretical value of x .

The discrepancy in the last two values is evidently due to the accompanying nitrification in which ammonia formed undergoes change to nitric acid.

APPLICATION TO NITRIFICATION

I

Warrington (3) has made an experiment upon the growth of nitric acid in nitrification. By the application of the method above described to his result we find:

$$\log \frac{1.1}{A - 1.1} = K (62 - t_1)$$

$$\log \frac{9.6}{A - 9.6} = K (77 - t_1)$$

$$\log \frac{13.1}{A - 13.1} = K (81 - t_1)$$

$$\log \frac{18.7}{A - 18.7} = K (96 - t_1)$$

$$\frac{9.6 (A - 1.1)}{1.1 (A - 9.6)} = 15 K$$

$$\frac{18.7 (A - 13.1)}{13.1 (A - 18.7)} = 15 K$$

$$\frac{9.6 (A - 1.1)}{1.1 (A - 9.6)} = \frac{18.7 (A - 13.1)}{13.1 (A - 18.7)}$$

$$A = 19.2$$

$$\frac{A}{2} = 9.6$$

$$t_1 = 77.$$

Then the equation becomes:

$$\log \frac{x}{19.2 - x} = K (t - 77)$$

By calculation of K from the observations at $t = 62, 81$ and 96 we have, respectively, $0.0811, 0.0830$ and 0.0828 . The mean of these values is 0.0823 . Hence the equation becomes:

$$\log \frac{x}{19.2 - x} = 0.0823 (t - 77)$$

Now giving t the value of $62, 77, 81$ and 96 we can calculate their corresponding theoretical value of x , as will be seen in Table IV in which the observed and calculated values of x are compared.

TABLE IV
COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	N as HNO ₃ per million	
	Found	Calculated
62	1.1	1.06
77	9.6	9.60
81	13.1	13.08
96	18.7	18.69

Owing to the fact that the experiment was made at intervals too infrequent to enable us to determine A , the number in parenthesis, therefore, was measured by the author from the curve and added.

The agreement between the observed and calculated values is quite good.

II

Applying the same method to another result of nitrification by Warrington we find also that the theoretical values of x nearly coincide with those of observation. Using the points ($t = 22, 35, 52$ and 65) on the curve to determine A , we have:

$$A = 25.3$$

$$\frac{A}{2} = 12.7$$

$$t_1 = 50.$$

Then the equation becomes:

$$\log \frac{x}{25.3 - x} = K (t - 50)$$

Giving t all of the observed values the average value of K is calculated to be 0.0501. Hence the equation becomes:

$$\log \frac{x}{25.3 - x} = 0.0501 (t - 50)$$

Now giving t the values of 22, 35, 49, 52 and 65 we find that the corresponding theoretical values of x are 0.96, 3.81, 11.92, 14.10 and 21.49, respectively. In Table V the observed and calculated values are compared:

TABLE V
COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	N as HNO_3 per million	
	Found	Calculated
22	0.7	0.96
35	2.0	3.81
49	11.9	11.92
(52)	14.2	14.10
65	20.1	21.49

III

Applying the same method to one other experiment on nitrification obtained by Lemmermann, Fischer and Heintz (1), we find good agree-

ment between the values of observation and calculation. Using the points ($t = 0, 14, 28$ and 42) on the curve for the determination of A we have:

$$A = 15.53$$

$$A$$

$$- = 7.77$$

$$2$$

$$t_1 = 13.$$

Assuming that the values obtained are correct, the equation becomes:

$$\log \frac{x}{15.53 - x} = K(t - 13)$$

Now when $t = 0$ we have:

$$\log \frac{4.70}{10.83} = -13K$$

whence $K = 0.0279$, similarly from the observations at $t = 14, 28, 42$ and 49 we find, respectively, $K = 0.0236, 0.0353, 0.0316$ and 0.0345 . The average value of K from these observations is 0.0305 , hence the equation becomes:

$$\log \frac{x}{15.53 - x} = 0.0305(t - 13)$$

Now giving t the values of $0, 14, 28, 42, 49$ and 77 , we can compute the corresponding theoretical value of x . In Table VI the theoretical and experimental values are compared.

TABLE VI
COMPARISON OF AMOUNT OF NITRATE NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Nitrate Nitrogen mg.	
	Found	Calculated
0	4.70	4.45
14	7.97	8.03
28	11.99	11.51
42	13.85	13.74
49	14.69	14.30
77	14.26	15.35

The agreement between calculated and actual values of nitrate nitrogen is as perfect as in the previous cases.

SUMMARY

The preceding discussion may be summarized as follows:

1. The processes of ammonification and nitrification are autocatalytic chemical reactions.

2. In ammonification and nitrification the maximum increase of ammonia and nitric acid in a unit of time occurs when the total amount of production due to these processes is half completed.

3. Increases of ammonia and nitric acid in the processes are in accordance with the formula

$$\log \frac{x}{A-x} = K(t-t_1)$$

where x is the amount of ammonia and nitric acid which has been produced at time t , A is the total amount of ammonia and nitric acid produced during the process, K is a constant and t_1 is the time at which half of the total amount of ammonia and nitric acid is produced.

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THE VERTICAL DISTRIBUTION OF PHOSPHORUS IN THE SURFACE SOIL OF PRAIRIES¹

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INTRODUCTION

The distribution of phosphorus in the successive horizontal layers of the soil is not governed by any universal rule (7, p. 252), the surface soil being sometimes richer and sometimes poorer in this constituent than the corresponding subsoil, as Wohltmann pointed out in connection with a study of the soils of western Germany (9, p. 31). In the Chernozem soils of Russia, according to Kossowitsch (6, p. 327), the amount in the first four to eight inches is somewhat higher than in the underlying layers.

The prairie soils of Illinois (5, p. 82-87) and Iowa (4, p. 138-152) show in most cases a higher percentage in the surface than in either the subsurface or the subsoil, the sections being, respectively, 0 to 6 2/3, 6 2/3 to 20 and 20 to 40 inches.

A recent study of the soils on different parts of the loess in Nebraska (1, p. 302) has shown that in general the proportion in the first and the second foot is smaller than that in the lower layers, and further that when the first is compared with the second foot, now the one and now the other contains the more.

It appears probable that any regularities found in the vertical distribution of phosphorus on the prairie soils of the loess would occur also in any other secondary soil that was originally uniform throughout the surface and lower layers, such as the soil on till plains and lacustral deposits like the clay loams of the Red River Valley, provided that the soil, since its deposition, had borne only a grassland vegetation.

EXPERIMENTAL

Having available a set of inch sections from the surface foot of the same fields on the Nebraska loess which had been sampled for the above-mentioned study, we have determined the total phosphorus by digestion with hydrofluoric and nitric acids (8, p. 163). With most of the samples the datum is the result of a single determination.

The soil samples (2, p. 206) had been collected from 30 virgin prairie fields, five near each of six stations of the United States Weather Bureau—Wauneta, McCook, Holdrege, Hastings, Lincoln and Weeping Water—ranging in annual precipitation from 18.5 to 30.2 inches, and in altitude from 3000 to 1100 feet, the first mentioned station being about

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300 miles west of the last. In each field 20 (and in the fields near Lincoln 50) cores 1 foot long and $1\frac{3}{8}$ inches in diameter were taken and subdivided into inch sections. Accordingly, each of the samples prepared for analysis, by combining those from the five fields of an area, was a composite of 100 (or 250) individual samples. In the case of the surface inch section all aerial portions of living plants had been removed but the roots as well as the plant debris upon the surface were treated as integral parts of the soil.

In calcareousness the soils of the different areas show a wide range (3, p. 414). The subsoils at McCook and Wauneta were very calcareous, while those at Lincoln and Weeping Water contained little carbonate. The surface foot in all the fields showed only very small amounts of carbonates, although apparently enough to meet fully the requirements of the most calciphilous of the ordinary farm crops—alfalfa.

It seems safe to assume that the twelve composites from any one of the six areas represent material which at the time of its deposition was uniform in composition, and, accordingly, that any important differences between these now found are to be attributed to subsequent alteration, due to leaching, the action of vegetation, etc.

The data are reported as phosphoric acid in Table I. In all six areas there is a decrease in the phosphorus content from the first to the twelfth inch. In the case of each area there is at least one section that shows a little more or less than corresponds to its position, but as in most cases the datum is the result of a single determination it is not improbable that most, if not all, of these slight irregularities in the steady decrease of phosphorus from the surface downward would have disappeared if duplicate determinations had been made. In the eastern three areas the first section, which carried considerable plant debris, is distinctly richer in phosphorus than the second, while in the western three areas there is little difference. When the distribution is compared by 3-inch, instead of by 1-inch, sections each of the areas shows a regular decrease from the surface downward, and when the upper half of the foot is compared with the lower half, the former shows from 8 to 30 per cent the more.

As in each area the organic matter also decreases steadily throughout the surface foot (2, p. 228) it is of interest to compare the change in the phosphoric acid content with that of the organic matter. In Table II are shown, for the 3-inch sections, the organic matter calculated from the organic carbon ($\text{Organic C} \times 1.724$) as determined by combustion with copper oxide (2, p. 228), and the ratio of organic matter to total phosphoric acid. The ratio decreases from the surface downward and from east to west. Here, as in so many other comparisons (1; 2; 3), the six areas form three groups: the easterly, distinctly humid two, Lincoln and

Weeping Water, showing a decrease of from 25 to 30 per cent; the western, distinctly semi-arid two, McCook and Wauneta, with almost 50 per cent; and the remaining two, occupying an intermediate position. Thus the concentration of phosphorus in the upper layers appears far less marked than that of the organic matter. While the upper layers are the richer in both, the accumulation of the one proceeds independently of the other. In confirmation of this, we may point out that a comparison of the amount of phosphoric acid (1, p. 302) in the surface foot of each of the

TABLE I
PHOSPHORIC ACID IN THE INCH SECTIONS OF THE SURFACE FOOT OF THE
DIFFERENT AREAS

Depth Inches	Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water	Average
	%	%	%	%	%	%	%
1	.150	.140	.178	.150	.168	.178	.161
2	.146	.146	.175	.137	.146	.158	.151
3	.156	.143	.165	.130	.141	.156	.148
4	.143	.146	.165	.131	.130	.150	.144
5	.146	.140	.165	.129	.135	.154	.145
6	.149	.138	.154	.125	.129	.151	.141
7	.133	.137	.131	.118	.127	.146	.132
8	.134	.134	.130	.118	.127	.138	.130
9	.127	.127	.130	.118	.124	.138	.127
10	.134	.131	.133	.108	.119	.129	.126
11	.137	.138	.129	.108	.121	.121	.126
12	.137	.124	.124	.101	.116	.124	.121
Average							
1-3	.151	.143	.173	.139	.152	.164	.153
4-6	.146	.141	.161	.128	.131	.152	.143
7-9	.131	.133	.130	.118	.126	.141	.130
10-12	.136	.131	.129	.106	.119	.125	.124
Average							
1-6	.148	.142	.167	.133	.141	.158	.148
7-12	.133	.132	.129	.112	.122	.133	.127
Average							
1-12	.141	.137	.147	.122	.132	.145	.137

five fields of any one area with that of the organic matter (2, p. 227), shows that the fields richest in the one constituent are liable to be poorest in the other. Accordingly, if in any one of the six areas, we sampled a large number of upland fields and arranged them in the order of their content of organic matter, we should not expect their relative richness in phosphorus to be at all definitely related to this arrangement. However, in the case of each of the fields we should expect to find the successive 3-inch layers of the surface foot to show decreasing amounts of both constituents.

When comparisons are to be made of the amounts of phosphorus in virgin with those in long-cultivated soils, or even in different virgin fields with one another, this marked difference in the phosphorus content of the different layers of the surface foot requires that special attention should be given to the details of sampling. For example, in the case of two fields, one of which has been used only as meadow while the other has for some years been used as a pasture, the latter may have suffered such

a compacting of the surface layers that an instrument designed to sample to just 6 inches will take from it a distinctly greater amount of soil, with the result that it will show a relatively smaller content of phosphorus. In careful studies of changes in composition induced by cultivation, close pasturage, etc., the relative densities of the different samples should be taken into consideration, as has previously been emphasized in connection with nitrogen studies (2, p. 221-225).

TABLE II
RATIO OF ORGANIC MATTER TO PHOSPHORIC ACID IN THE 3-INCH SECTIONS OF
THE SURFACE FOOT

ORGANIC MATTER							
Depth Inches	Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water	Average
	%	%	%	%	%	%	%
1-3	3.91	3.60	6.30	5.90	6.70	6.59	5.50
4-6	2.65	2.88	3.81	3.54	4.99	4.91	3.80
7-9	2.19	2.32	2.94	2.90	4.14	4.02	3.08
10-12	1.84	1.85	2.58	2.59	3.42	3.68	2.66
1-6	3.28	3.24	5.05	4.72	5.84	5.75	4.65
7-12	2.01	2.08	2.76	2.74	3.78	3.85	2.87
1-12	2.65	2.66	3.90	3.73	4.81	4.80	3.76
RATIO OF ORGANIC MATTER TO PHOSPHORIC ACID							
1-3	26.0	25.2	36.3	41.9	43.9	40.0	35.5
4-6	18.1	20.4	23.5	27.5	38.0	32.5	26.7
7-9	16.6	17.5	22.5	24.6	32.8	28.6	23.8
10-12	13.5	14.1	20.1	24.4	28.8	29.5	21.7
1-6	22.0	22.8	29.9	34.7	40.9	36.2	31.1
7-12	15.0	15.8	21.3	24.5	30.8	29.0	22.7
1-12	18.5	19.3	25.6	29.6	35.8	32.6	26.9

An explanation of the concentration of the phosphorus in the uppermost layers of the natural grassland soils does not seem difficult to find. The plant roots forage in both surface soil and subsoil, finding the phosphoric acid in the latter as readily available as in the former, or in some cases even more so (1, p. 306-312). The greater portion of that entering the roots will be deposited in the aerial parts of the plants, and later, on the death of the leaves and stems, be left upon the surface. Each succeeding year will witness a new draft upon the phosphorus of the subsoil without any return. The surface nine inches holds the bulk of the roots, with their contained phosphoric acid, partly drawn from the underlying layers, and the nearer the surface the different 3-inch layers are, the more of the plant debris will they receive through surface fissures and the work of insects.

The accumulation of phosphorus in the surface layers of a soil should not be expected to account for all that has been removed from the corresponding subsoil. Water and wind cause a more or less constant ero-

sion of the superficial phosphorus-rich soil layer and the plant debris and their deposition in valleys or partial removal to the sea.

The influence the wild herbivorous animals may have exerted upon the distribution of the phosphorus merits separate consideration. It is probable that in many species the carcasses with their accumulation of phosphorus were not distributed over upland and valley at all uniformly. In this connection we might allude to the tendency at the present time of the cattle on the ranges of the west to be overtaken by death, in seasons of great drouth, near streams or water-holes, and during unusual winter storms, in sheltered spots.

SUMMARY

Prairie loess soils in the surface foot show a steady decrease in phosphorus from the surface inch downward, independent of the aridity of the climate in which they have formed.

The concentration of phosphorus in the surface layers, while it may be attributed to the prairie vegetation, is not dependent upon a corresponding concentration of organic matter, the difference between the first and the twelfth inch being much the greater with the latter.

In taking samples for comparisons of the phosphorus content of long-cultivated with virgin prairie fields or even of prairie fields with one another it is important to take into consideration the relative densities of the soils.

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JAPANESE LABORATORY PORCELAIN WARE

TRADE MARK "S. C. P."

We continue to recommend JAPANESE LABORATORY PORCELAIN WARE, trade mark "S. C. P.," as the best porcelain ware now available. This statement is based, not only on the unanimous satisfaction expressed by users of the ware for the past year—we have received twenty-two shipments since October, 1915—but upon the results of the following laboratory tests. These tests have been directed to ascertain:

1. The effect of chemical reagents.
2. The behavior of the glaze at high temperatures.
3. Constancy of the weight of the vessels.
4. Resistance to sudden changes of temperature.

TEST 1. To Determine the Effect of Chemical Reagents.—The three reagents which are most likely to attack porcelain ware were employed, i. e.,—

1. Strong Sulphuric Acid (Sp. Gr. 1.84) at boiling point.
2. 10% Solution of Caustic Soda at 90°-100° C.
3. 10% Solution of Sodium Carbonate at 90°-100° C.

Each of these tests was continued for four hours. Concentration was prevented in the case of Caustic Soda and Sodium Carbonate by conducting the experiment in a closed oven which prevented evaporation. From the loss in weight sustained by the dish and the area wet, the loss per 100 sq. cm. was calculated.

RESULT

With strong Sulphuric Acid loss per 100 sq. cm.—0.00103 gram.
With 10% Caustic Soda Solution loss per 100 sq. cm.—0.0104 gram.
With 10% Sodium Carbonate Solution loss per 100 sq. cm.—0.00103 gram.

TEST 2. To Determine the behavior of the Glaze at High Temperatures.—A dish containing small pieces of broken porcelain from other dishes was subjected to about 1000° C. in a gas muffle for a period of four hours.

RESULT

A slight discoloration took place but the glaze was unaffected and, while pieces of broken dishes adhered to the glaze, they were easily removed by slight pressure of the fingers, leaving the surface unbroken. It will be noted that this test was made very severe and more likely to bring about the destruction of the dish than will be met with in general work.

TEST 3. To Determine the Constancy of the Weight.—For this test crucibles were carefully heated and then weighed. They were then heated for one and one-half hours in a muffle at a temperature of 850° C.

RESULT

Five crucibles gave an average increase in weight of 0.0014 gram. This increase in weight was accompanied by a marked discoloration of the porcelain.

TEST 4. To Determine the Resistance to Sudden Changes of Temperature.—A dish was filled with wax which was melted and heated gradually to a temperature of 160° C. The source of heat was then removed and the dish containing the molten wax was allowed to cool slightly until the temperature of the wax had fallen to 150° C. This procedure was adopted so that the temperature of the dish should equal that of the wax and not higher. When the specified temperature had been attained the dish was plunged into water at 15° C. As it remained unbroken, the test was repeated at temperatures up to 230° C. Dishes were also put in a muffle previously heated to about 850° C. and when hot were taken out with cold iron tongs and placed on a cold surface.

RESULT

In the first test the dishes remained unbroken when heated to a temperature of 230° C. and in the second test were placed on a cold surface without cracking or chipping of the glazed surface.

The above tests, which were conducted in accordance with the specifications of the National Physical Laboratory in some recently published tests for British porcelain ware, do not sufficiently emphasize the property of great resistance to sudden temperature change possessed by the Japanese porcelain as is shown by the following practical tests we recently conducted in a nearby laboratory:

A No. 0 tall form crucible, 15cc. capacity, was heated red hot over a Bunsen burner and placed successively while red hot, and after reheating each time, on a copper plate chilled with ice and on a cake of ice. The crucible was reheated over a Bunsen burner while still wet from the ice and only cracked when this operation had been repeated three times.

In the same way an evaporating dish No. 5, capacity 300 cc., was filled with water, placed directly over a Bunsen burner, water evaporated to dryness and the dish transferred immediately to a cold iron plate and into cold water without breakage.

In another dish were placed iron drillings with strong Nitric and Sulphuric Acid which was evaporated to fuming Sulphuric Acid and the dish then plunged into cold water without breakage.

The above are practical tests for heat shock which can be readily confirmed in any laboratory.

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